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Effectiveness of intranasal lysine-aspirin in patients with aspirin-sensitive, and aspirin tolerant nasal polyposis: controlled trials

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Thesis submitted to the University of London
For the degree
Doctor of Philosophy
In
Otorhinolaryngology

2006

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For Apurva & Saroj

Acknowledgements

The successful completion of this body of work, which primarily involved clinical trials supported by laboratory-based analysis, required help from numerous individuals. Naming each person would require more than a few pages. Hence, I would like to say a collective thank you to everyone who helped me with these projects.

However, in most research projects there are always certain individuals who deserve a very special note of thanks. I would like to single out Glenis (Scadding) who not only gave me the opportunity for this research but also has been and still is a source of constant encouragement. I am deeply indebted to her for the constant guidance, supervision and support.

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Lastly, I would like to thank the nursing staff at RNTNE hospital, Ray Allen, my Registrar colleagues, and friends outside of work – Sushrut, Mallika, Kailash, Vinay, David, Nick, Sanjay, Mus, and Sujeet.

Effectiveness of intranasal lysine-aspirin in patients with aspirin-sensitive, and aspirin tolerant nasal polyposis: controlled trials

Abhijeet Apurva Parikh

(Abstract)

Therapeutic options for patients with aspirin-sensitive, and aspirin tolerant nasal polyps include corticosteroids (topical and systemic), surgery to relieve nasal obstruction, or a combination of the two. Medical therapy i.e. corticosteroids aims to reduce the underlying inflammation and alleviates all nasal symptoms. The aim of surgery is to provide an adequate nasal airway for breathing.

Intranasal lysine-aspirin has been used as an option in these patients, and trials have shown its beneficial effects. However, their design and interpretation have been open to scientific criticism. Therefore, we planned to study the effectiveness of intranasal lysine-aspirin in the two groups of polyp patients by conducting trials in a randomised, double blind, and placebo controlled manner. Diagnosis of aspirin-sensitivity or tolerance was confirmed by intranasal lysine-aspirin challenge prior to enrolment in the appropriate trials. Nasal biopsy and polyp tissue were collected from the patients for laboratory-based experiments. Analysis of their results was aimed at improving our understanding of aspirin-sensitivity, and to generate a hypothesis on its pathogenesis.

Intranasal lysine-aspirin did not reduce polyp growth, or improve nasal symptoms in aspirin-sensitive or aspirin tolerant patients when compared to placebo. However, intranasal lysine-aspirin (16 mgs) did not have any deleterious effects in both groups. Immunohistochemistry revealed a significant increase in expression of $CysLT_1$ receptor on inflammatory cells in nasal biopsies from aspirin-sensitive compared to tolerant patients. This expression reduced following treatment with intranasal lysine-aspirin, which suggested a possible mechanism of desensitization. We also found significantly higher levels of iNOS activity in polyp tissue from aspirin-sensitive compared to tolerant patients.

Enhanced expression of $CysLT_1$ receptor provides further evidence of the central role played by leukotrienes in aspirin-sensitivity. Also, we suggest that high iNOS activity in polyp tissue is secondary to increased leukotriene production, and that the latter is most likely to be confined to the respiratory mucosa of patients with aspirin-sensitive asthma and/or polyposis.

Declaration

The work included in this thesis involved 2 clinical trials, and 2 collaborative projects. Dr. Glenis Scadding and Professor Tak Lee were responsible for the conception of the trial on aspirin-sensitive patients. I was substantially involved in the design, and conduct of the trial. I undertook the analysis, and interpretation of generated data.

Dr. Glenis Scadding was responsible for the conception of the trial on aspirin tolerant patients. I was substantially involved in the design and conduct of the trial, its analysis and in the interpretation of generated data.

Dr. Ana Sousa, who worked as a scientist in Professor Lee's department of Respiratory Medicine & Allergy, King's College London, performed immunohistochemistry of nasal biopsies from aspirin-sensitive and aspirin tolerant patients. I took the nasal biopsies and was responsible for their labeling and storage. Immunohistochemical analysis was part of our trial in aspirin-sensitive nasal polyp patients.

The other collaborative project (iNOS expression in nasal polyps) was with Professor Jane Mitchell, Department of Pharmacology in Critical care, National Heart & Lung Institute. I was substantially involved in the conception of the project, its analysis, and interpretation.

I take full responsibility for interpretation of findings from the trials and studies that have been included in this thesis, as well as the ensuing discussion.

CONTENTS

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ABBREVIATIONS

General:

AA	Arachidonic acid
Amin	Minimum cross-sectional area (mm^2)
AR	Acoustic rhinometry
AS	Aspirin-sensitive (individuals or patients)
ASy	Aspirin-sensitivity
AT	Aspirin tolerant (individuals or patients)
B-LT	Leukotriene B receptor
COX	Cyclooxygenase
$CysLT_1$	Cysteinyl leukotriene receptor
DFP	5,5-dimethyl-3-[2-isopropoxy]-4-[methanesulfonylphenyl]-2[5*H*]-furanone (selective COX-2 inhibitor)
FEV_1	Forced expiratory volume in 1 minute
IFN	Interferon
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LAS	Lysine-aspirin
LO	Lipooxygenase
LT	Leukotriene
LTC_4S	Leukotriene C_4 synthase
mRNA	Messenger ribonucleic acid
NIPF or NF	Nasal inspiratory peak flow
NO	Nitric oxide
NSAIDs	Nonsteroidal anti-inflammatory drugs
PCR	Polymerase chain reaction
PEFR or PF	Peak expiratory flow rate
PG	Prostaglandin
PLA_2	Phospholipase A_2
QOL	Quality of life
RNTNEH	Royal National Throat, Nose & Ear Hospital
TX	Thromboxane

UPSIT	University of Pennsylvania Smell Identification test
VAS	Visual analogue scale
Vol	Volume (mls)

Units of measurement:

mg(s)	milligram (plural)
μg(s)	microgam (plural)
mm	millimetre
ml	millilitre
min	minute

Statistical terms:

S.D	Standard deviation

LIST OF FIGURES

Figures are labelled according to chapter, and number. Therefore, figure 1 in chapter 1 is labelled Figure 1.1. Every endeavour has been made to have a figure on the page following its citation in the text.

LIST OF TABLES

Tables are labelled according to chapter, and number. Therefore, table 1 in chapter 1 is labelled Table 1.1. Every endeavour has been made to have a Table on the page following its citation in the text.

LIST OF APPENDICES

These are included at the end of the thesis following references.

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   Leukotriene receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis.
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2. A Sousa, A Parikh, G K Scadding, C Corrigan, T H Lee.
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   Clinical profile of aspirin-sensitive and aspirin-tolerant nasal polyposis, and results of intranasal lysine-aspirin challenge.
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INTRODUCTION

1.1 Aspirin

1.1.1 *General facts*

Aspirin or acetylsalicylic acid has been available commercially for over 100 years. Bayer & Co. first marketed the drug in 1899 (Vane, 2000). Yearly world production of aspirin is estimated at 100,000 tons (Vane, 1976). In the UK, the average consumption of aspirin is 100 tablets/head/year. Low-dose aspirin, as a prophylactic against vascular events has gained popularity, and approximately 6% of 60-year-old subjects consume 300 mgs of it at least 5 days/week (Weil et al. 1995).

1.1.2 *History*

Rev. Edward Stone in 1763 was the first to report on the therapeutic effects of salicylates for acute fevers (Vane, 1976). Aspirin was developed at Bayer & Co. by Felix Hoffman, a young Chemist, and Dr. Heinrich Dresser who was head of the research department (Vane, 1976). Hoffman's father used salicylic acid for his arthritis but was troubled by gastric upset following its use. Thus, it was acetylated in the hope that this would reduce its gastric irritability. As a consequence acetylsalicylic acid was born. Salicylic acid came from Spirea plants (spiric acid, or spirin). Therefore, acetylsalicylic acid became acetylspirin or Aspirin.

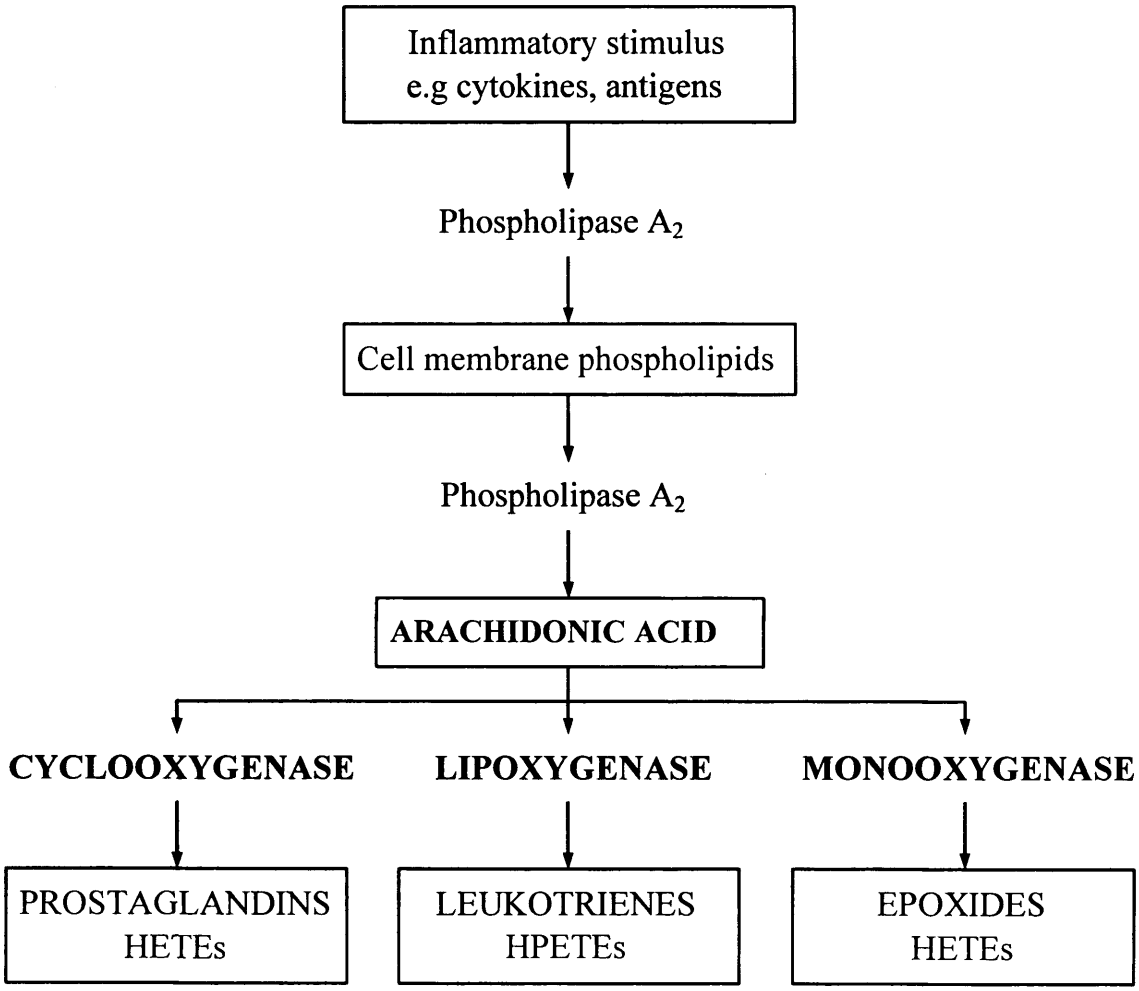
1.1.3 *Mode of action of aspirin and other Non-steroidal anti-inflammatory drugs (NSAIDs)*

1.1.3a Arachidonic acid metabolism

A variety of stimuli e.g. antigens, cytokines, can activate signal transduction pathways that culminate in activation of phospholipase A_2 (PLA_2). This enzyme cleaves arachidonic acid (AA) from cell membrane phopholipids. Oxygenation of AA produces a variety of physiologically active mediators, which play an important role both in

Figure 1.1

Pathways of Arachidonic acid metabolism



HETE: Hydroxyeicosatetraenoic acid
HPETE: Hydropeoxyeicosatetraenoic acid

'housekeeping', and inflammation (Figure 1.1). This occurs via 3 enzymatic pathways – cyclooxygenase, lipooxygenase, and monooxygenase (Holtzman, 1991). Two important pathways are briefly summarised below.

Cyclooxygenase pathway (Figure 1.2):

Two chemical reactions are responsible for metabolism of AA down this pathway. Oxygenation inserts 2 molecules of oxygen into AA forming prostaglandin (PG) G_2. It is reduced by the peroxidase activity of cyclooxygenase (COX) to PGH_2, which forms the substrate for the production of active metabolites – Thromboxane, Prostacyclin, and Prostaglandins (PGD_2, PGE_2, $PGF_{2\alpha}$).

Lipooxygenase pathway (Figure 1.3):

Three different lipooxygenases (LO) – 5, 12, and 15 metabolise AA. Of these, 5LO products are called Leukotrienes (LTs). Biologically active leukotrienes are LTC_4, LTD_4, and LTE_4. These cysteine-containing LTs play a role in immediate hypersensitivity reactions, and also have pro-inflammatory effects (Samuelsson, 1983).

1.1.3b Action of aspirin/NSAIDs

NSAIDs were discovered in the latter half of the 20th century. Despite their different chemical structures they had identical therapeutic effects, and adverse reactions as aspirin. Thus, it was clear that their mode of action would be alike. In 1971 Vane (Vane, 1971) demonstrated that aspirin like drugs inhibited prostaglandin synthesis in a dose dependent manner, and this was supported in two further studies published simultaneously (Ferreira et al. 1971; Smith and Willis, 1971). Vane (Vane, 1971) postulated that it was inhibition of prostaglandin synthetase (cyclooxygenase – COX), which prevented prostaglandin formation. Subsequently it was shown that acetylation of prostaglandin synthetase by aspirin like drugs prevented the synthesis of prostaglandins (Roth et al. 1975).

Figure 1.2

ARACHIDONIC ACID METABOLISM

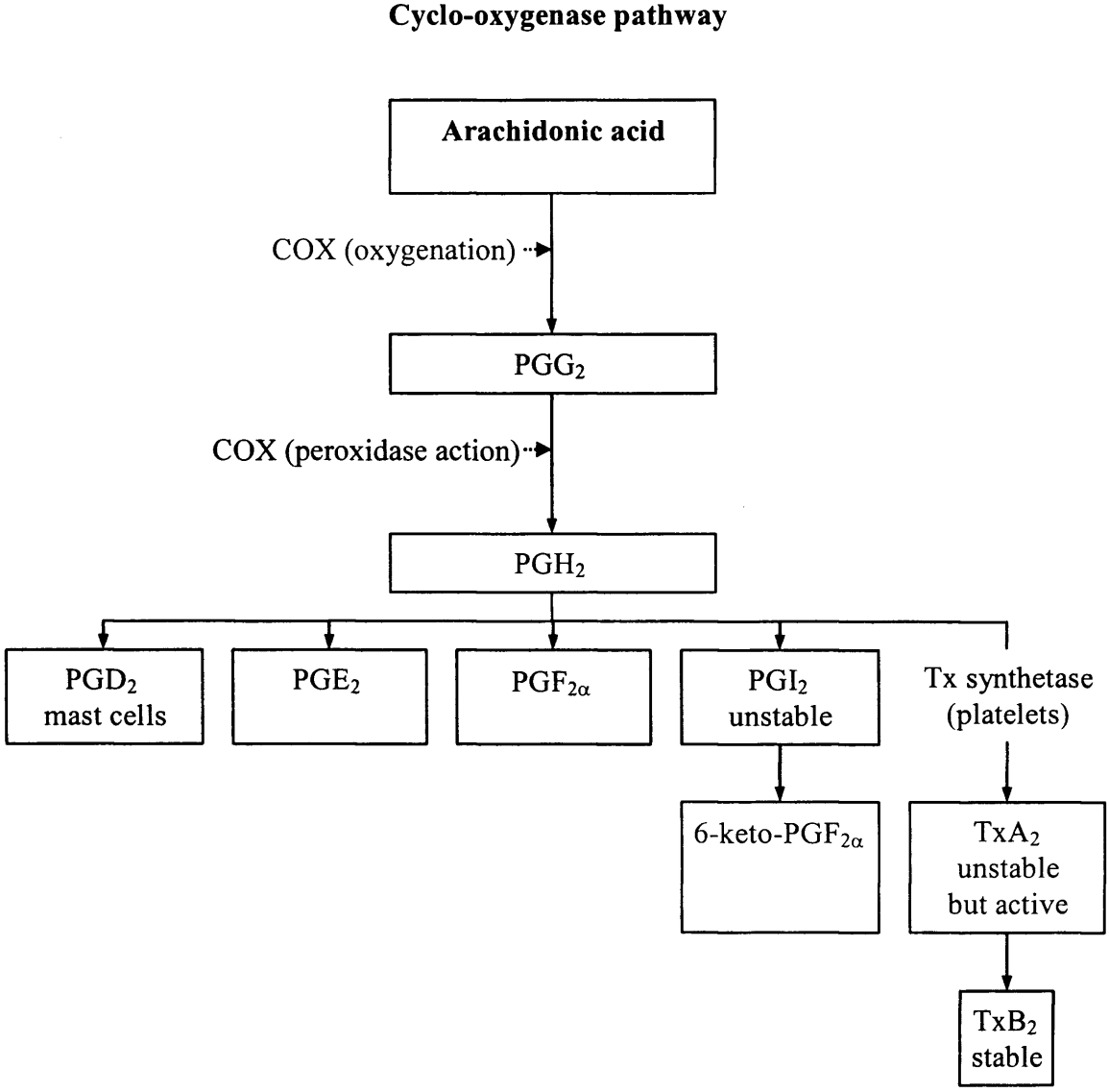
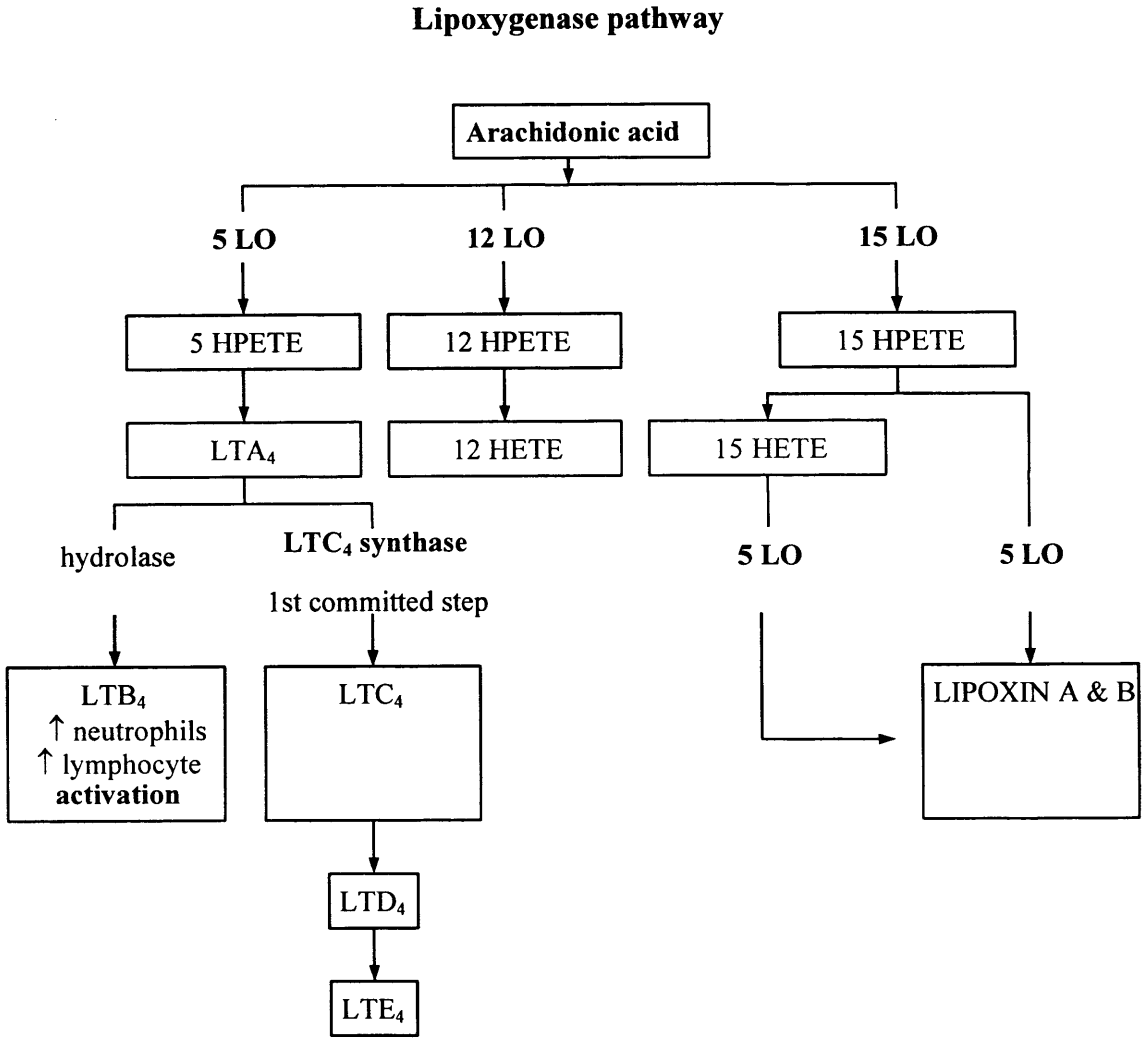


Figure 1.3

ARACHIDONIC ACID METABOLISM



1.1.3c COX-1/COX-2/COX-3

Cyclooxygenase (COX) is present in two isoforms in the body (Vane, 2000). These are termed COX-1 and COX-2. The former is a constitutive isoform found in most tissues, whereas the latter is an inducible isoform produced in inflammatory settings. Aspirin modifies COX-1 and COX-2 resulting in their *irreversible* inhibition. NSAIDs cause a *reversible* inhibition. The therapeutic effects, and adverse reactions of aspirin/NSAIDs are said to be due to this inhibition of COX-1/2, which in turn prevents prostaglandin formation. It is now widely accepted that the side effects of aspirin/NSAIDs are due to constitutive COX-1 enzyme inhibition (Warner et al. 1999), whereas the beneficial effects are due to inducible COX-2 enzyme inhibition. This knowledge has fuelled the research to find pharmacological agents with selective COX-2 inhibitory properties. Recently a third isoform, COX-3 has been isolated, which may be selectively inhibited by drugs like acetaminophen (Chandrasekharan et al. 2002).

1.1.4 *Non-steroidal anti-inflammatory drugs (NSAIDs)*

These drugs are organic acids that cause a competitive reversible inhibition of cyclooxygenase (Table 1.1) (Insel, 2001). Thus, their duration of action is dependent on their pharmacokinetic clearance from the body. As mentioned above, new selective COX-2 inhibitors are being manufactured that have a reduced incidence of side effects, in particular gastrointestinal ulceration.

1.1.5 *Therapeutic uses/Side effects of Aspirin & NSAIDs*

Aspirin and NSAIDs as a group are classically used as antipyretics, analgesics, and anti-inflammatory agents. However, reports of other beneficial effects of low dose aspirin continue to appear in literature (Symmons, 1996). It is used for secondary prevention of myocardial infarcts, stroke, vascular dementia, and there is some evidence of it reducing the risk of colorectal cancer. Other uses of NSAIDs include in neonates with patent

Table 1.1

Classification of analgesic, antipyretic, and non-steroidal anti-inflammatory drugs

Non selective COX inhibitors	*Salicylic acid derivatives:* Aspirin Sodium salicylate Diflunisal Sulfasalazine *Para-aminophenol derivatives:* Acetaminophen *Indole and Indene acetic acids:* Indomethacin Sulindac *Heteroaryl acetic acids:* Diclofenac Ketorolac *Arylpropionic acids:* Ibuprofen Naproxen Ketoprofen *Anthranilic acids:* Mefenamic acid *Enolic acids:* Piroxicam Meloxicam *Alkanones:* Nabumetone
Selective COX-2 inhibitors	*Diaryl substituted furanones:* Rofecoxib *Diaryl substituted pyrazoles:* Celecoxib *Sulfonanilides:* Nimesulide

ductus arteriosus (PDA), and in women with primary dysmenorrhoea. With the advent of selective COX-2 inhibitors the side effects will reduce considerably, and potential uses will include premature labour, colon cancer, and Alzheimer's disease (Vane, 2000). Gastrointestinal ulceration and bleeding is the main side effect of aspirin and NSAIDs. The risk of such occurrence is 3 times that of nonusers (Insel, 2001). It is due to inhibition via cyclooxygenase blockade (COX-1 & 2) of prostaglandin synthesis (PGI_2, PGE_2), which are cytoprotective to the gastric mucosa. Recently introduced selective COX-2 inhibitors are considerably safer in this respect. The other side effect of note is bruising secondary to reduced platelet aggregation due to inhibition of thromboxane (TX_2) formation. Aspirin is particularly notorious because of its irreversible cyclooxygenase blockade.

1.1.6 *Intolerance to Aspirin and NSAIDs*

Certain individuals react in an abnormal manner to low or therapeutic doses of aspirin or NSAIDs. Terms used to describe this reaction include 'aspirin-sensitivity', 'aspirin-intolerance', and 'aspirin-idiosyncrasy'. In this thesis the term aspirin-sensitivity (ASy) will be used, and the individuals will be called aspirin-sensitive (AS). There are 3 kinds of reactions:

Respiratory reaction: Majority of the individuals intolerant to aspirin/NSAIDs will have this type of reaction (80%). Typically it occurs 20-30 minutes after aspirin/NSAID intake. Reaction can occur anytime from 10 minutes to 2 hours. Patients have an attack of asthma. The attack can be severe and unresponsive to conventional medication with the patient requiring hospitalisation, and needing intensive care admission. Fatalities have been reported. These individuals may have a nasal reaction, with rhinorrhoea and blockage being the main symptoms. A small subset of individuals have a reaction that affects only the nasal lining with sneezing, itching, rhinorrhoea, and nasal obstruction.

Skin reaction: Some individuals (10%) develop urticaria and/or angioedema. Unlike the respiratory reaction it can occur up to 14 hours after aspirin/NSAID intake. Facial swelling particularly around the eyes and lips is seen. Occasionally the oedema is severe so as to involve the tongue and larynx. This can culminate in death.

Combined reaction: There are individuals (10%) who have both the above-mentioned reactions to varying degrees after aspirin/NSAID use.

This aspirin-sensitivity is different from patients who have an anaphylactic reaction to the drug (Berkes-Eva, 2003). Here the patient goes into shock almost immediately following aspirin ingestion.

1.2 Aspirin-sensitivity

1.2.1 *Early case reports*

The first case of aspirin-sensitivity to be reported in medical literature was in 1902, three years after aspirin appeared on the market (Hirschberg, 1902). The patient had an urticarial reaction, and purpura after taking 1 gm. of aspirin. Gilbert described the case of a 40-year-old woman with chronic asthma who developed severe urticaria, which he called 'urticaria gigantean or angioneurotic oedema' after taking 300 mg of aspirin (Gilbert, 1911). She was treated with epinephrine and made a full recovery. Although there is no mention of wheezing in the report, it was concluded that the patient's symptoms represented an attack of asthma, which was aggravated by an 'intense idiosyncrasy' to aspirin.

1.2.2 *Early case series*

As Physicians became more aware of this occurrence, they started to group cases together in order to present common features and also speculate on the underlying mechanism of the adverse reaction. Three such series are discussed here.

Cooke reported on a collection of 15 cases (Cooke, 1919). Nine cases (60%) developed asthma, and 3 (20%) had urticaria after aspirin intake. It is not clear from the paper if the remaining 3 had a combination of reactions. The primary aim of this series report was to comment on the possible underlying mechanism of these side effects. Cooke described idiosyncrasy to a drug as an 'exaggerated normal or side action'. This he thought could be due to various reasons, one being an unstable mechanism by which a drug acts in the body. However, for aspirin he suggested 'allergy' as a reason because the adverse reactions i.e. asthma or urticaria were not its 'exaggerated normal or side action'. In fact in 1919 the definition of allergy was far from our present day understanding of the subject. Anaphylaxis was considered an antigen-antibody reaction whereas an allergy was 'the natural hypersensitiveness of the individual not produced by immunologic processes, as the exciting agents or allergens are in many cases not capable of producing antibodies'.

Lamson and Thomas described 4 cases, all of them women (Lamson and Thomas, 1932). They highlighted the widespread use of aspirin, and also the possibility of its

inadvertent intake in nostrums (quack remedies), which were inadequately labeled. It seems likely that one of the cases had an anaphylactic reaction as the symptoms occurred within minutes of taking aspirin. Two of the cases are of note because nasal symptoms are mentioned. One of them suffered from nasal obstruction along with asthma, and the other noticed an exacerbation of hay fever- like symptoms every time she took aspirin.

Prickman and Buchstein studied 62 cases of aspirin-sensitivity, and published their findings in the first comprehensive clinical review (Prickman and Buchstein, 1937). They noted a high incidence amongst asthmatics, and quoted Van Leeuwen's estimate, which was about 10%. It is also stated that the incidence is much higher in patients with more severe asthma. Clinical observations in their 62 patients (Table 1.2) included a female preponderance (M:F = 1:2), age distribution (66% = between 31-50 years), presence of asthma in 43 patients (69%), history of urticaria/angioneurotic oedema in 10 patients (16%), vasomotor rhinitis in 21 patients (34%), hay fever in 11 patients (17%), and nasal polyps in 21 patients (34%). They administered a tablet of aspirin (an oral challenge), and studied various aspects of the adverse reaction. Thirty patients (48%) reacted within 1 hour, the mean time being 32 minutes. Asthma was the most frequent reaction occurring in 61% of cases, and urticaria/angioedema in 19%. The others had a combination of reactions. Three patients stated that their first attack of asthma was precipitated by aspirin ingestion. Again, it is brought to our attention that patients can inadvertently take aspirin as part of an unlabelled proprietary preparation.

1.2.3 *Samter and Beers clinical study*

Over a 10-year period (1954-1965) Samter and Beers followed more than 1000 patients with aspirin-sensitivity (Samter and Beers, 1968). From this cohort 182 patients were prospectively studied, and their observations formed the basis of the paper. The clinical features of this group are very similar to patients from Prickman's series (Table 1.2).

Of particular note however, are the observations made on the natural history of this condition. The majority of the patients (80%) followed a particular pattern for development of their respiratory tract disease. The initial indication is the development of profuse watery rhinorrhoea in the 2nd or 3rd decades of life. At first this is intermittent but it gradually leads to chronic nasal blockage. This is suggestive of polypoid degeneration, which was present in 50% of their cases.

Table 1.2

Clinical features of aspirin-sensitive patients

Clinical feature	Reference	
	Samter (1968) n = 182	**Prickman (1937) n = 62**
Age in years		90%: 21-60
Sex: male (female)	78 (104)	22 (40)
Age at onset	≤ 30: 46 (25%)	21-50: 63%
History of asthma		43 (69.3%)
Family history of atopy	40 (22%)	32 (51.2%)
Nasal polyps	92 (50%)	21 (33.9%)
Types of reaction:		
• **Asthma**	154 (85%)	38 (61.2%)
• **Urticaria/angioneurotic oedema**	18 (10%)	12 (19.3%)
• **Asthma and urticaria/angioneurotic oedema**	10 (5%)	2 (3.2%)

Asthma started in middle age in most of their patients. Episodes in many individuals were precipitated after a polypectomy. Respiratory tract disease continued despite aspirin avoidance, and interestingly in some patients their first aspirin induced reaction was a few years after nasal polyps and asthma developed.

Commenting on the triad of nasal polyps, asthma, and aspirin intolerance they report that 'it is a disease entity, not a chance clutter of allergic symptoms, and represents, in fact, the prototype of a syndrome that has not been previously described and deserves recognition'. Thus, it has been suggested by many that this triad should be called Samter's syndrome (Probst et al. 1992). However, the above comment may not be accurate as this triad has been recognised since the early part of the 20th century (Widal et al. 1987).

A further aspect highlighted in this paper was the cross-reactivity of certain foodstuffs, and food colourings in this group of patients. Twenty-six patients (14%) had reacted to foodstuffs and analysis revealed that the most common preservative used was sodium benzoate while the colouring was tartarazine or Food, Drug & Cosmetic yellow no.5 (FDC yellow no.5).

1.2.4 *Epidemiology*

The frequency of aspirin-sensitivity in the population has been estimated to be between 0.3-0.9% (Settipane, 1983; Settipane et al. 1974; Settipane et al. 1980). The figure is higher in patients with rhinitis, asthma, nasal polyps, and urticaria (Table 1.3, Figure 1.4). A recent study from Finland estimates the prevalence of doctor-diagnosed aspirin intolerance to be 5.7% (Hedman et al. 1999).

In patients with nasal polyps the prevalence varies from 3-26% (Table 1.4). Studies relying on history tend to underestimate the prevalence. Higher estimates are seen in studies that have a larger proportion of asthmatics, which may indicate a greater severity of disease. A reasonable estimate of 11% comes from a large study of 1600 patients with nasal polyps (Brown et al. 1979).

Conversely, the prevalence of nasal polyps in patients with aspirin-sensitivity is between 36-72% (Mygind, 1999). A recent study conducted by the European Network on Aspirin-Induced Asthma (AIANE) quotes a figure of 60% (Szczeklik et al. 2000). However, this estimate can rise to near 100% if endoscopy and CT scanning of sinuses is performed (Mullol et al. 2001).

Table 1.3

Prevalence of aspirin-sensitivity (%) in various conditions

Condition	Reference	
	Settipane (1983)	Hedman (1999)
Normal population	0.3	1.2
Rhinitis	1.4	2.6
Asthma	4-19	10.7
Nasal polyps	14-23	27.9
Urticaria	23-28	5.2

Figure 1.4

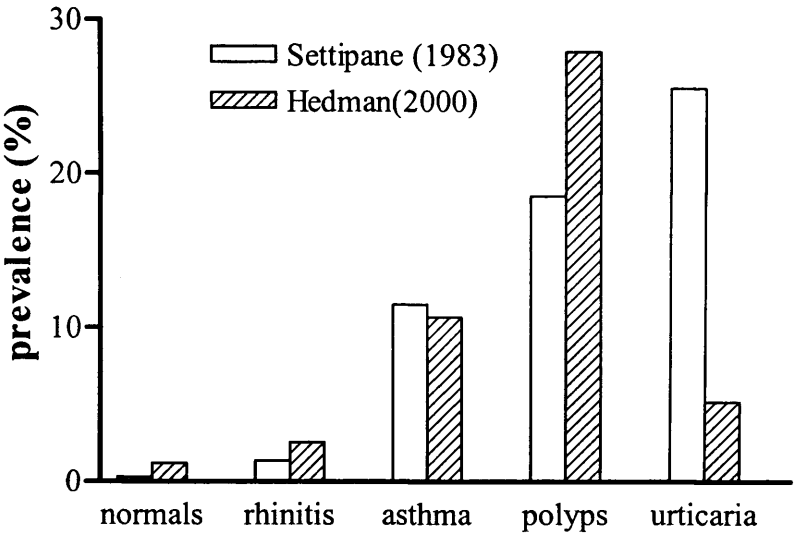


Table 1.4

Prevalence of aspirin-sensitivity (AS) in nasal polyp (NP) patients

Author	Year of publication	NP (n=)	% AS
Delaney	1976	100	3
Moloney	1977	445	6
Drake-Lee et al	1984	200	6
Brown et al	1979	1600	11
Larsen et al	1994	103	13
Schenck	1974	174	14
Settipane	1987	211	14
Jantii-Alanko et al	1989	85	26

1.2.5 *The nose in aspirin-sensitive patients*

1.2.5a Natural history of nasal disease

Rhinitis characterized by watery discharge, sneezing, and intermittent nasal obstruction appears in the 2nd decade of life (Samter and Beers, 1968). Nasal blockage becomes persistent and is accompanied by anosmia. Approximately 2 years later the patients have their first attack of asthma. The first attack of aspirin-sensitivity occurs about 4 years later and around the same period in time nasal polyps are diagnosed. This classical thinking of disease progression has been debated (Szczeklik et al. 2000). With modern techniques of examination like rigid nasendoscopy it is likely that polyps could be discovered prior to the patients first attack of asthma or aspirin sensitivity.

1.2.5b Nasal polyps in aspirin-sensitive patients

Macroscopically the appearance of polyps is similar in aspirin-sensitive and aspirin-tolerant patients. They are pedunculated smooth masses that are pale, semi translucent, bilateral, and project into the nasal cavity from the middle meatus. This is a functional and anatomical area on the lateral wall of the nose where the sinuses drain. Inflammation giving rise to the polyps is not confined to the lining of the ethmoid sinuses but also involves the frontal, sphenoid, and maxillary sinuses.

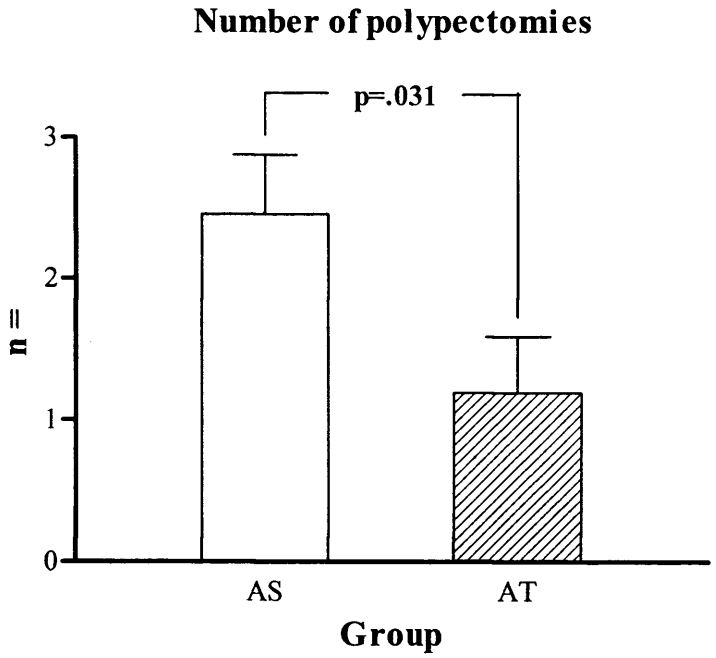
Nasal polyp disease is more aggressive in aspirin-sensitive as compared to aspirin-tolerant patients (Settipane et al. 1991). Aspirin-sensitivity is a highly significant factor affecting the recurrence rate (Drake-Lee et al. 1984). A study on the recurrence of nasal polyps provides good evidence of this aggressiveness (Jantti-Alanko et al. 1989). Of the aspirin-sensitive group 59% required repolypectomies compared to 19% of the aspirin-tolerant group. Also, the majority of aspirin-tolerant patients had needed 1 polypectomy compared to aspirin-sensitive patients who had undergone 3 or more polypectomies. Other studies have highlighted the same issue (Table 1.5; Figure 1.5).

Table 1.5

Number of polyp operations in aspirin-sensitive (AS) vs. aspirin tolerant (AT) patients

Study	Year	Group	
		AS: mean (n)	AT: mean (n)
Drake Lee et al	1984	3.27 (11)	1.4 (172)
English	1986	1.8 (205)	
Schenck	1974	3.94 (18)	1.92 (156)
Szczeklik	2000	2.6 (500)	
Larsen et al	1994	1.86 (7)	1.39 (84)
Jantti-Alanko et al	1989	1.23 (22)	.05 (63)

Figure 1.5



1.2.5c Inflammatory cells and Mediators in Aspirin-sensitive nasal disease

i) Nasal polyps

Microscopically polyps from aspirin-sensitive and aspirin-tolerant patients appear similar. Typically there is marked stromal oedema, infiltration with inflammatory cells, thickening of the basement membrane, and local fibrosis (Hamilos, 1996). However, closer examination of the cellular infiltrate and mediator content reveals several differences.

All types of *inflammatory cells* are found in nasal polyps (Takasaka et al. 1986). However, there is a preponderance of eosinophils and mast cells. In a quantitative study using immunohistochemistry, polyps from aspirin-sensitive patients were found to have 920/mm^2 eosinophils compared to 520/mm^2 in patients with asthma plus nasal polyps, and 880/mm^2 in patients with only nasal polyps (Jankowski et al. 1989). Mast cells numbered 40/mm^2, 20/mm^2, and 20/mm^2 respectively. Other studies analysing eosinophil/mast cell characteristics found evidence of increased activation (Ogata et al. 1999), and degranulation (Takasaka et al. 1986; Yamashita et al. 1989). In addition to enhanced activation there is evidence showing increased survival. An interesting recent study shows that the number of apoptotic eosinophils in nasal polyps was significantly lower in aspirin-sensitive patients (mean±S.E.M, 5.5±1.5/mm^2) compared to atopic (18.7±3.8/mm^2), and non-atopic patients (21.3±5.2/mm^2) (Kowalski, 2000).

Studies on *mediator content* of nasal polyps have shown 2 main differences between aspirin-sensitive and aspirin tolerant patients. The first is low histamine content in aspirin-sensitive patients compared to aspirin tolerant patients (Bumsted et al. 1979; Hosemann et al. 1990). In one study the histamine content of nasal polyps from aspirin-sensitive asthmatics was 6.13μg/gm as opposed to 25.31μg/gm in patients without aspirin-sensitivity or asthma (Bumsted et al. 1979). The second difference is in the levels of arachidonic acid metabolism products. Two studies have shown that the ratio of cyclooxygenase/lipoxygenase products is significantly lower in aspirin-sensitive patients compared to aspirin tolerant patients (Pinto et al. 1997; Yamashita et al. 1989). Thus, polyps from aspirin-sensitive patients have high levels of leukotrienes (Table 1.6, Figure 1.6). Also, there is a significantly lower amount of PGE_2 in polyps from aspirin-sensitive as opposed to aspirin tolerant patients (Kowalski et al. 2000; Schmid et al. 1999; Yamashita et al. 1989).

Table 1.6

Arachidonic acid metabolites in nasal polyp tissue

(from Yamashita T, et al. *Rhinology* 1989; suppl 8:15-24)

Group	Prostaglandins (10^{-9} g/g tissue)				Leukotrienes (10^{-12} g/g tissue)	
	E_2	$F_{2\alpha}$	6-keto-$PGF_{1\alpha}$	TxB_2	C_4	D_4
CS	33.30±8.78	4.07±2.51	3.74±1.03	14.1±7.29	172±50	242±107
AR	28.6±10.21	6.45±3.23	4.56±2.13	8.96±6.84	336±327	211±158
ASA	7.33±2.28	1.45±0.66	3.77±2.94	4.56±1.48	1831±1719	1019±692

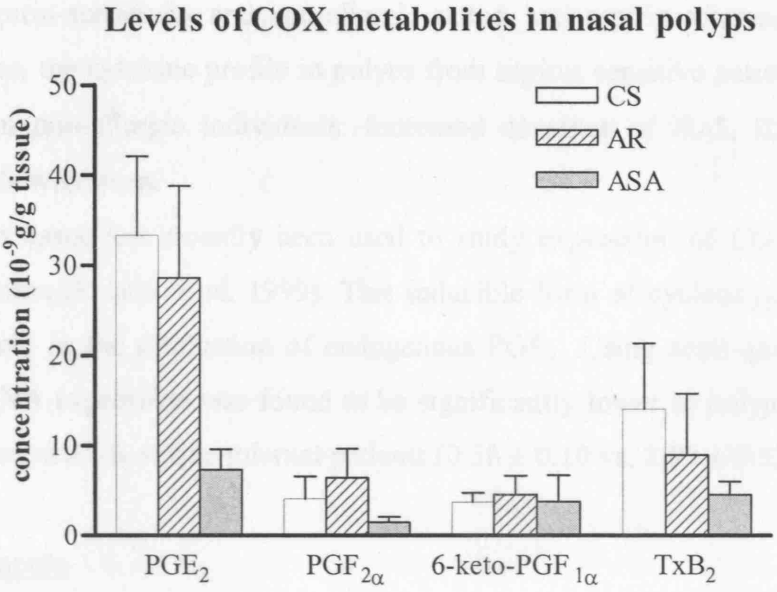
Key: CS= chronic sinusitis (n=5); AR= nasal allergy (n=5); ASA= aspirin-sensitive asthma (n=5)

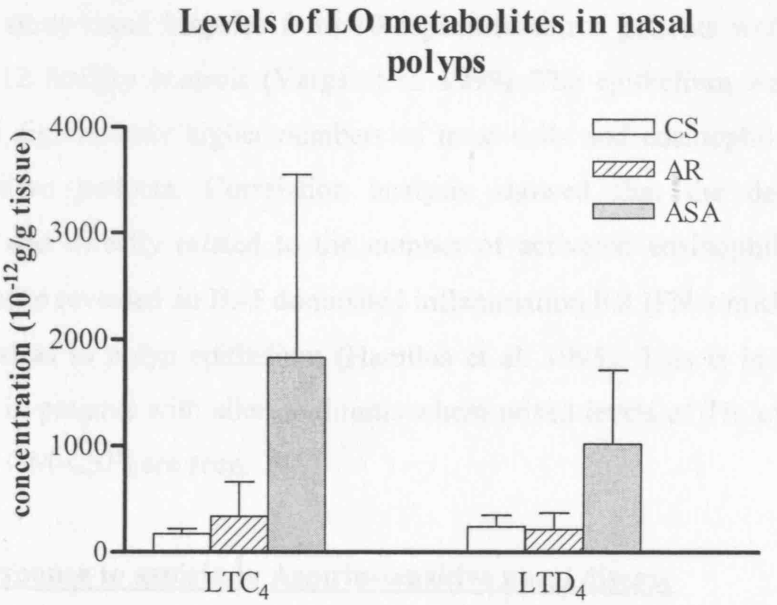
Figure 1.6

Arachidonic acid metabolites in nasal polyp tissue

(from Yamashita T, et al. *Rhinology* 1989; suppl 8:15-24)

Key: CS= chronic sinusitis (n=5); AR= nasal allergy (n=5); ASA= aspirin-sensitive asthma (n=5)





Further differences have also been noted on *mediator release* following addition of aspirin to polyp tissue in-vitro. Cultured polyp epithelial cells from aspirin-sensitive patients when incubated with aspirin were found to generate very high levels of 15-hydroxyeicosatetraenoic acid (15-HETE), whereas no effect was seen on 15-HETE generation by cells from aspirin tolerant patients (Kowalski et al. 2000).

Cytokine expression is also different in aspirin-sensitive and aspirin tolerant patients (Hamilos et al. 1995). Correlation statistics revealed a significant positive relationship between aspirin-sensitivity and non-allergic status, and aspirin tolerance and allergic status. Hence, the cytokine profile in polyps from aspirin-sensitive patients was similar to that from non-allergic individuals. Increased densities of IL-5, IL-2, and IFN-γ $mRNA^+$ cells were seen.

Nasal polyp tissue has recently been used to study expression of *COX-2* isoform of cyclooxygenase (Picado et al. 1999). This inducible form of cyclooxygenase plays an important role in the production of endogenous PGE_2. Using semi-quantitative PCR, COX-2 mRNA expression was found to be significantly lower in polyps from aspirin-sensitive compared to aspirin tolerant patients (0.38 ± 0.10 vs. 2.93 ± 0.52).

ii) Nasal mucosa

Compared to nasal polyp studies, the mucosa of the nose has been studied infrequently. In a recent study nasal biopsies from 10 aspirin-sensitive patients were compared to those from 12 healthy controls (Varga et al. 1999). The epithelium was considerably thicker, and significantly higher numbers of mast cells and eosinophils were seen in aspirin-sensitive patients. Correlation analysis showed that the degree of nasal obstruction was directly related to the number of activated eosinophils. Analysis of cytokine profile revealed an IL-5 dominated inflammation but IFN-γ $mRNA^+$ cells were not increased as in polyp epithelium (Hamilos et al. 1995). This is in contrast to the profile seen in patients with allergic rhinitis where raised levels of Th_2 cytokines (IL-4, IL-5, IL-13, GM-CSF) are seen.

iii) Nasal response to aspirin in Aspirin-sensitive nasal disease

Nasal lavage has been widely used to study the response of the nose to either oral aspirin (Ferreri et al. 1988; Fischer et al. 1994; Kowalski et al. 1993) or its intranasal

instillation (Kowalski et al. 1996; Picado et al. 1992). In one study oral aspirin ingestion led to an increase in LTC_4 and histamine levels (Ferreri et al. 1988). However, this was only seen in patients who had both a naso-ocular and bronchospastic reaction. Those who had only a bronchospastic reaction did not show the rise in mediators. Other studies have shown that the rise is not confined to LTC_4 levels, but involves all cysteinyl leukotrienes (Kowalski et al. 1993; Picado et al. 1992). Also, the rise in these mediator levels seems to coincide with nasal symptoms (Ferreri et al. 1988; Kowalski et al. 1993). The source of these mediators appears to be eosinophils and mast cells (Fischer et al. 1994; Kowalski, 1996). Influx of eosinophils associated with a marked rise in eosinophil cationic protein, indicative of activation, has been shown. Tryptase levels also rise, which indicates mast cell degranulation.

1.2.6 *Mechanism of aspirin-sensitivity*

Three important events have greatly contributed to our present day understanding of aspirin-sensitivity. In 1967 a report appeared in medical literature which linked indomethacin, a NSAID, to aspirin-sensitivity (Vanselow and Smith, 1967). This link was confirmed the same year in another observational study (Samter and Beers, 1967). Eighteen hospitalised aspirin-sensitive patients were given 25 mgs each, precipitating an attack of asthma. Thus, it became evident that these two drugs with varied chemical structures caused similar adverse reactions in aspirin-sensitive individuals. The second event was a landmark study published in 1971, proposing that the mechanism of action of aspirin-like drugs was through inhibition of prostaglandin synthesis (Vane, 1971). The last event that significantly helped our understanding of aspirin-sensitivity was the discovery of leukotrienes as products of arachidonic acid metabolism along the lipooxygenase pathway (Samuelsson, 1983). In the first 70 years of the 20^{th} century most observers thought, and tried to prove that aspirin-sensitivity was an immune mediated phenomenon. However, the above-mentioned events led to a shift in thinking towards a biochemical alteration, and most recent studies have concentrated on investigating these changes. A summary of the 2 periods is provided below.

1.2.6a. Immunologic studies

The nature of adverse reactions i.e. acute asthma, naso-ocular reaction, urticaria, angioedema, and their rapid onset after aspirin intake led most clinicians to believe that aspirin-sensitivity was an immune-mediated event. However, studies aimed at demonstrating such a relationship could not prove so or were inconclusive.

Skin tests were used to try and demonstrate the immunologic theory but the complete lack of a reaction led to alternative ways of explaining the link (Cooke, 1922; Freidlaender and Feinberg, 1947). One theory was that aspirin acted like a hapten combining with a serum protein subsequently leading to the adverse reaction (Bruce Pearson, 1963; Landsteiner, 1924). To demonstrate such a possibility 12 aspirin-sensitive patients were skin tested with salicylates containing human sera (Mathews et al. 1950). None of the patients had a positive response. Another theory suggested a reaction between aspirin and an antibody generated due to an extraneous antigen such as an infectious agent (Feinberg and Malkeil, 1951). Further in-vitro studies using conjugates of aspirin and serum protein also failed to prove a specific immunologic event (Giraldo et al. 1969; Yurchak et al. 1970).

This lack of evidence led to various non-immunologic theories. One theory suggested the presence of abnormal nasal/pulmonary kinin receptors in aspirin-sensitive patients, which were stimulated by aspirin intake (Samter and Beers, 1967). This theory was particularly attractive because by then it was known that chemically unrelated compounds such as indomethacin caused the same reactions in these individuals. Another suggestion was a direct stimulation of the complement system by aspirin (Yurchak et al. 1970). However, around this time the mechanism of action of aspirin and NSAIDs was discovered and the emphasis shifted towards a biochemical theory.

1.2.6b Biochemical studies

In 1975 a study was published firmly implicating many more NSAIDs to aspirin-sensitivity (Szczeklik et al. 1975). Eleven aspirin-sensitive asthmatics were given a variety of NSAIDs orally in gradually increasing doses. A reduction of 15% in peak flow rate was taken as a positive response. Also, the ability of these NSAIDs to inhibit prostaglandin synthetase (cyclooxygenase) in-vitro was studied. It became apparent that the capability of these drugs to induce bronchospasm was directly proportional to their

power in inhibiting prostaglandin synthetase activity. Despite these observations it was difficult to explain why the majority of asthmatics were tolerant to NSAIDs. The investigators proposed that inhibition of COX prevented the formation of prostaglandins E and F, which paved the way for endogenous histamine to act as a potent bronchoconstrictor. In aspirin tolerant patients the action of histamine was combated by the β-adrenergic system thus preventing bronchoconstriction, but in aspirin-sensitive patients the β-adrenergic system did not play a similar role. Another explanation came in 1977 suggesting NSAID induced COX inhibition lead to a preferential lack of bronchodilator PGE_2 over bronchoconstrictor $PGF_{2\alpha}$ thus, provoking asthma (Toogood, 1977).

The discovery of additional pro-inflammatory derivatives of arachidonic acid, which were named leukotrienes (Samuelsson, 1983), led to a shift in aspirin-sensitivity research towards studying these new mediators. In particular, the emphasis was on investigating the role of cysteinyl leukotrienes LTC_4, LTD_4, LTE_4, as they were potent bronchoconstrictors. In the early days of leukotriene directed research an in-vitro study showed that there was a 3-fold increase in antigen-provoked leukotriene production by bronchial tissue following incubation with indomethacin (Undem et al. 1987). Thus, the possibility arose that inhibition of COX lead to an increased generation of leukotrienes. In aspirin-sensitive individuals an increase in leukotriene output after intake of aspirin or NSAIDs was confirmed by studies measuring levels in nasal lavage (Ferreri et al. 1988), urine (Christie et al. 1992; Christie et al. 1991), monocytes (Juergens et al. 1992), and bronchoalveolar washouts (Sladek et al. 1994). Also, there was evidence suggesting an elevated baseline metabolism of AA in these patients (Christie et al. 1991; Israel et al. 1993; Juergens et al. 1992). These observations generated a popular theory on the causation of aspirin-sensitive disease, called the 'shunting hypothesis'. However, aspirin or NSAIDs should also cause a similar 'shunting' of AA towards the 5LO pathway in aspirin tolerant individuals with enhanced production of LTs. This is not seen, calling into question the validity of this hypothesis (Mitchell and Belvisi, 1997).

Researchers were aware of this dilemma (VanArsdel, 1984), and an additional hypothesis was proposed suggesting increased target organ responsiveness to leukotrienes in aspirin-sensitive individuals (Stevenson, 1987). In a study conducted to test this hypothesis, 5 aspirin-sensitive patients and 15 aspirin tolerant patients were

challenged with histamine or LTE_4 (Arm et al. 1989). In aspirin-sensitive patients LTE_4 was found to be 1870 times more potent than histamine in causing a 35% reduction in airway conductance. In addition, the mean dose of LTE_4 required to reduce airway conductance was 16.5 times more in aspirin tolerant as opposed to aspirin-sensitive patients (0.17nmol vs. 2.8nmol). Thus, the study confirmed that aspirin-sensitive patients demonstrated increased airway responsiveness to leukotrienes. Another study confirmed the pivotal role played by 5LO products in aspirin-sensitive patients (Israel et al. 1993). In this study 8 aspirin-sensitive patients were pre-treated with a 5LO inhibitor (Zileuton) and challenged with oral aspirin in a double-blind, placebo-controlled, crossover manner. After Zileuton, the baseline urinary LTE_4 levels decreased, the rise after aspirin was not as steep, and it prevented the occurrence of nasal symptoms.

Other observations prompted studies into the role that PGE_2 may play in aspirin-sensitive patients. PGE_2 levels are significantly low in polyps from aspirin-sensitive patients (Kowalski et al. 2000; Schmid et al. 1999; Yamashita et al. 1989). It is a known bronchodilator (Mathe and Hedqvist, 1975), and in an early study on asthmatics it was shown to prevent bronchospasm caused by oral aspirin in a subgroup of aspirin-sensitive individuals (Pasargiklian et al. 1977). This phenomenon has been confirmed by the other studies (Sestini et al. 1996; Szczeklik et al. 1996).

A significant finding that implicated leukotrienes as the primary culprits in aspirin-sensitive disease came from a study that showed a profound overexpression of LTC_4 synthase (LTC_4S) in bronchial tissue from aspirin-sensitive patients (Cowburn et al. 1998; Sampson et al. 1997). Biopsies from aspirin-sensitive patients had 5 times the cells staining for LTC_4S compared to aspirin tolerant patients, and 9 times compared to normal individuals. The cells showing enhanced expression were eosinophils. These findings were followed by genetic work, which suggested that a polymorphism in the promoter region of the gene coding for LTC_4S might represent a risk factor to NSAIDs in patients with asthma (Sanak et al. 2000; Sanak et al. 1997). A single nucleotide transversion from adenine to cytosine found 444 nucleotides upstream from the first codon in the LTC_4S gene was associated with a relative risk of 3.89 for development of a aspirin-sensitive phenotype. However, this polymorphism could not be demonstrated within aspirin-sensitive patients in the United States (Van Sambeek et al. 2000). The study concluded that finding the $_{-444}C$ allele might represent a population-stratified polymorphism seen in patients of eastern European descent.

1.2.7 *Diagnosis of aspirin-sensitivity*

In patients with nasal polyps, and/or asthma, it is important to determine their aspirin-sensitive status as it has prognostic and therapeutic significance. This is generally done by taking a history and direct questioning. However, the only method of confirming aspirin-sensitivity is by undertaking a challenge test. Most commonly these are employed in research studies on aspirin-sensitive individuals. Rarely they may be undertaken in patients where the history is equivocal and aspirin or NSAIDs are required for treatment of other conditions such as rheumatoid arthritis or heart disease.
Oral aspirin challenge was used for most of the last century, but it can precipitate a severe reaction. Techniques studied as an alternative to oral challenge in the hope that they would prove to be safe and reliable, included skin tests with aspirin (Cooke, 1922; Duke, 1933; Prickman and Buchstein, 1937), aspirin containing serum (Mathews et al. 1950), and aspirin-protein conjugates (Yurchak et al. 1970). All these were negative. Other inconclusive methods were immunoflourescent test and haemagglutination testing (Yurchak et al. 1970), or studying histamine release from nasal polyps of aspirin-sensitive patients (Hosemann et al. 1990). Methods used commonly are discussed below.

1.2.7a History

All patients with nasal polyps and/or asthma should be asked about aspirin-induced reactions. Typical clinical characteristics seen in aspirin-sensitive patients have been mentioned in the first four sections of this chapter. The likelihood of aspirin sensitivity is greater in patients with both nasal polyps and asthma (Settipane, 1983), or those who have required frequent hospitalisation for control of their asthma (Szczeklik et al. 2000). Patients with frequent nasal polyp recurrences needing surgery are also more likely to be aspirin-sensitive (Settipane et al. 1991).
Unless a patient gives a definite history linking aspirin intake with an adverse reaction, typical in its clinical and temporal characteristics, a reliable diagnosis of aspirin sensitivity cannot be made based on this method alone. Problems with history have been highlighted in previous studies. In one study 8.8% of individuals with a positive challenge were unaware that they had aspirin sensitivity, whereas 35% who thought they were aspirin-sensitive, had a negative challenge (Spector et al. 1979). In a more

recent investigation 15% of patients with a positive challenge were oblivious of their aspirin sensitivity (Szczeklik et al. 2000). Underreporting may be due to deliberate avoidance of NSAIDs as asthmatics may be aware of the potential for adverse reactions, or a lack of recognition of mild NSAID-induced symptoms.

1.2.7b Aspirin Challenge tests

Direct intake of aspirin as in a challenge test remains the only method to prove conclusively an individuals aspirin-sensitive/tolerant status (Szczeklik and Nizankowska, 2000). Aspirin has been administered via 3 different routes namely, oral, inhalation, and nasal.

i) Oral challenge test

Aspirin was first used to diagnose aspirin sensitivity in 1933 (Duke, 1933). A small granule of aspirin was placed on the tongue and the individual was watched over a period of 1 minute for development of symptoms. The temporal characteristics of an aspirin-induced reaction are such that this test would have been negative in the majority of aspirin-sensitive individuals. It was also regarded as unsafe.

A systematic approach to oral aspirin challenge was introduced in 1975 (Szczeklik et al. 1975). Patients were given gradually increasing doses of aspirin and other NSAIDs, which conclusively proved their cross-reactivity. However, a standardized protocol was suggested a few years later (Mathison and Stevenson, 1979). Informed consent was introduced, patients were warned of potential dangers, and intensive care facilities were available if needed. Challenge was not recommended in asthmatics with a 1-second forced expiratory volume (FEV_1) of less than 70% of predicted value. A challenge was considered as positive if patients developed naso-ocular symptoms or their FEV_1 dropped by 30%. Most challenges were undertaken in a single-blind, placebo-controlled manner. Patients were observed for a period of 4½ hours or in cases of urticaria for 24 hours. Two different schedules were used; a one-day test for patients with a history of weak reactions, and a two-day test for patients with more severe reactions. Despite the potential for serious adverse events an oral challenge still remains the 'gold standard' against which new challenge methods are compared. Recently oral aspirin challenge has been evaluated scientifically (Nizankowska et al. 2000). Considering a positive test to

be a fall in FEV_1 of over 20% and/or extrabronchial symptoms (nasal congestion, rhinorrhoea, redness of the face, eye congestion), the sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy were reported as 89%, 93%, 97%, 77%, and 90% respectively (Table 1.7).

ii) Inhalation challenge

It is also called bronchial provocation or challenge. This method was introduced in 1977 using lysine-aspirin, which is a truly soluble form of aspirin (Bianco et al. 1977). Also, indomethacin has been used (Martelli, 1979). Subsequently a standardized protocol for this method was put forward (Schmitz-Schumann, 1999). The method is safer and quicker than an oral challenge (Nizankowska et al. 2000; Phillips et al. 1989; Schapowal et al. 1995; Szczeklik and Nizankowska, 2000). The challenge is considered to be positive if the FEV_1 drops by 20%. Most reactions occur in 30-45 minutes, although observations should be continued for 3 hours as delayed reactions are seen occasionally. The study mentioned above also evaluated inhalation challenge (Nizankowska et al. 2000). The same criteria were used to denote a positive challenge. For this method the sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy were reported as 77%, 93%, 96%, 64%, and 82% respectively (Table 1.7).

iii) Nasal challenge test

This method of provocation was first used in 1987 to study release of mediators (Ortolani et al. 1987). As a diagnostic tool it was used unsuccessfully in 1989 (Clement et al. 1989). The authors concluded that a nasal challenge test was not a useful diagnostic method. However, they used aspirin in powder form for their challenge. It was not until the soluble form – LAS was used that positive results were obtained, and its value as a safe test established (Pawlowicz et al. 1991). An early study showed differences in sensitivity and specificity when the test was conducted in patients with aspirin-sensitive rhinitis or aspirin-sensitive asthma or aspirin-sensitive urticaria (Schapowal et al. 1995). The figures were 93%, 97% for aspirin-sensitive rhinitis, 76%, 97% for aspirin-sensitive asthma, and 8%, 100% for aspirin-sensitive urticaria. This indicated its unreliability in patients with urticaria. A more recent study refutes this

conclusion (Tomaz et al. 1997). It shows sensitivity/specificity figures to be 80%, and 100%. However, the test was conducted in a small number of patients (n=10).

Recently, lysine-aspirin nasal challenge has been evaluated in detail (Milewski et al. 1998). The maximum dose of LAS used was 16 mgs. After challenge, nasal flow was measured using anterior rhinomanometry (reduction of 40%) along with symptom scores and peak flow rate. The sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy are reported as 78%, 95.6%, 96.9%, 71%, and 84.4% respectively (Table 1.7). None of the patients had any reduction in FEV_1, reaffirming its safety. A drawback is its inconsistency in patients with almost complete nasal obstruction or those that have large fluctuations in nasal airflow measurements. To circumvent this problem acoustic rhinometry, which is another instrument for assessing nasal response has been used (Casadevall et al. 2000). Using a reduction of 25% in nasal volume as an end point, the sensitivity and specificity of the test was found to be 73% and 94% respectively. Most scientists conclude that nasal challenge is a simple, quick, and a safe test to undertake (Mullol et al. 2001).

1.2.7c In-vitro test

Topical methods of challenge i.e. inhalation and nasal are safer than an oral challenge, but not as sensitive. In addition, all these tests require patients to spend at least a day in the hospital. Thus, an in-vitro test would be advantageous. The cellular antigen stimulation test or CAST has been studied for this purpose (Czech et al. 1995; Kubota et al. 1997; May et al. 1999). In one study the release of cysteinyl leukotrienes by leukocytes from aspirin-sensitive and aspirin tolerant patients was measured (Czech et al. 1995). The C5a induced release of these mediators was higher in leukocytes from aspirin-sensitive patients. However, others point out that the test may be too cumbersome for routine use, and its sensitivity variable (Kubota et al. 1997). To improve the tests reliability leucocytes from aspirin-sensitive patients were incubated with various NSAIDs (May et al. 1999). Although the sensitivity reached a 100% in aspirin-sensitive patients with urticaria a figure of only 72% was achieved in aspirin-sensitive patients with respiratory reactions (Table 1.7). The problems outlined above have prevented its widespread application in clinical practice.

Table 1.7

Sensitivity and Specificity of diagnostic tests

Study	Type of challenge	Dose of lysine-aspirin (mgs)	Parameter measured	Results			
				Sen	Spec	PPV	NPV
Milewski et al (1998)	**Nasal**	16	AR + Symptoms	**78**	**95.6**	**96.9**	**71**
May et al (1999)	**In vitro test (CAST)**	-	-	**72.7**	**96.7**		
Casadevall et al (2000)	**Nasal**	25	AcR	**94**	**73**		
Nizankowska et al (2000)	**Oral**	10-500 (cumm. dose)	PEFR + Symptoms	**89**	**93**	**97**	**77**
	Inhaled	0.18-182 (cumm. dose)	PEFR + Symptoms	**77**	**93**	**96**	**64**
Schapowal et al (1995)	**Nasal**	2	AR	**93**	**97**		

Key:

CAST = cellular allergen stimulation test; cumm. = cumulative dose; AR = active anterior rhinomanometry; AcR = acoustic rhinometry; PEFR = peak expiratory flow rate (litres/min); Sen = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value.

1.2.8 *Management*

Aspirin-sensitive disease is a chronic inflammatory process affecting the airways and skin. Currently there is no cure for it and management of the patient involves treating its various manifestations. These are asthma, urticaria, and nasal polyposis. In the following subsections I will consider treatment options that impact on all 3, followed by each manifestation individually. Desensitization, which does impact on all aspects of the disease process is discussed in detail as a separate section.

1.2.8a Avoidance of aspirin/NSAIDs

Avoiding aspirin, NSAIDs, and aspirin containing remedies is a precautionary measure (Schapowal et al. 1995). It prevents an acute adverse reaction which may be potentially life threatening. It is important to emphasize to the patient that aspirin is part of many 'over the counter' medications e.g. flu remedies, and may be consumed inadvertently (Prickman and Buchstein, 1937). It is of paramount importance to check the contents of these medicines prior to consumption. However, avoidance of aspirin/NSAIDs does not alter the natural course of the disease process (Samter and Beers, 1968).

1.2.8b Diet

Certain food additives have aspirin like effects. These include food dyes, colourings, and preservatives such as benzoates (Samter and Beers, 1968). Provocation tests with these substances resulted in asthma or urticaria in 7 of 8 patients (Juhlin et al. 1972). A similar study in a larger number of patients provoked positive reactions in 70% of individuals (Genton et al. 1985). Many food additives have been shown in-vitro to have weak inhibitory effects on the cyclooxygenase system reducing thromboxane production and platelet aggregation (Williams et al. 1989). Thus, there appears to be experimental evidence to support dietary restriction measures.

The beneficial effects of a diet have been shown in several studies. One such study on patients with recurrent urticaria who had a positive provocation test with food additives showed that 24% were symptom free, 57% were better, and 19% were unchanged (Ros et al. 1976). The follow-up period ranged between 6-24 months, and

diet restriction was at least 1-3 months. Another such study included 24 patients with asthma and urticaria (Genton et al. 1985). These patients had positive provocation tests. Twenty patients (83%) noticed an improvement within 5 days of starting their exclusion diet, which persisted for 8-14 months. These included 6 of 9 (67%) patients with asthma and 14 of 15 (93%) with urticaria. Complete disappearance of symptoms was only seen in patients with urticaria. These clinical studies add further support to the role of an elimination diet in patients with aspirin-sensitive disease (Table 1.8).

1.2.8c Alternatives to aspirin/NSAIDs

Table 1.1 lists the various drugs that will cause an adverse reaction in aspirin-sensitive patients, and hence should be avoided. Thus, in these patients a safe alternative is required. Acetaminophen (Paracetamol) can be used, but cross-reactivity is seen in 34% of aspirin-sensitive individuals (Settipane et al. 1995). They found mild, easily reversible bronchospasm with a dose higher than 1 gram. If there is a doubt about a patient's tolerability to acetaminophen an oral challenge can be undertaken (Schapowal et al. 1995). In one study 95% of aspirin-sensitive patients who were tolerant to acetaminophen had a negative oral challenge 1-3 years later (Quarantino et al. 1997). This proved its long-term safety. In the small minority of patients with reactions to acetaminophen a safe alternative would be an opiate such as dextropropoxyphene. Oral challenge with this drug was negative in 77 aspirin-sensitive patients (Szczeklik et al. 1977). Nimesulide was a new NSAID in the early 1990s and was evaluated as an alternative in 429 aspirin-sensitive patients (Andri et al. 1994). Only 3.3% (11) had a positive oral challenge test with a therapeutic dose of 200 mg. The reason for its relative safety remained unclear until the discovery of COX-2, the inducible form of the enzyme, and its preferential inhibition by Nimesulide (Famaey, 1997). With the discovery of COX-2 (Vane, 2000), selective COX-2 inhibitors have been introduced into the market. Their tolerability has been shown in various studies (Quarantino et al. 2000; Sanchez Borges et al. 2001; Szczeklik et al. 2001). Rofecoxib seems to be safest and this may be related to its highly specific ability of inhibiting COX-2.

Table 1.8

Dietary measures in management of aspirin-sensitive patients

(from Genton C, et al. *J All Clin Immunol* 1985; 76:40-45)

FOODS PERMITTED		TO AVOID
Cereals	Bread, and all cereals bought in fresh state	Coloured toothpaste, coloured cosmetics, coloured beverages, wines, alcohol, artificial sweeteners, ice cream, sweets, and commercially available desserts
Fats	Butter, olive oil	
Fruits	All permitted if in moderate amounts	
Meats	Fresh meat, pork, eggs; fish in small quantities only	
Vegetables	All (in fresh state) except cabbage, beans, spinach, sauerkraut, tomatoes only moderately	
Condiments	Sugar, salt, pepper, others only as dried leaves, vinegar without additives	
Sweets	Homemade without additives	
Beverages	Fresh milk, tea, coffee, homemade fruit juice, mineral water	

1.2.8d Antileukotriene drugs

The key role played by leukotrienes, particularly cysteinyl leukotrienes, in aspirin-sensitive disease has been highlighted in the section on mechanism. Therefore inhibition of leukotriene production should have a major impact on this disease process. Two ways of preventing the physiological action of leukotrienes are interference with their generation (5LO inhibition), or with their binding at receptor level. In the early 1990s the availability of pharmacological agents possessing these abilities made it possible to conduct several experimental studies to evaluate their effect in aspirin-sensitive patients (Nasser and Lee, 1997). In a study using a weak cystLT$_1$ antagonist, partial inhibition (47%) of the bronchoconstrictor response to inhaled lysine-aspirin was shown (Christie et al. 1991a). A more potent antagonist caused a 4.4 fold right-directed shift in the dose-response curve following inhalation of lysine-aspirin to induce bronchoconstriction compared to a placebo (Dahlen et al. 1993a). The same group, studying the same drug, also found a bronchodilator response (mean increase in FEV$_1$= 18%) in aspirin-sensitive patients, which lasted 9 hours (Dahlen et al. 1993b).

Other studies evaluated a 5LO inhibitor, Zileuton. Patients pre-treated with this drug in a double-blind, placebo-controlled design, were shown to have reduced baseline urinary LTE$_4$ production (Israel et al. 1993). In addition, it also prevented the development of nasal and dermal symptoms when challenged with aspirin. However, a recent study suggests that it does not inhibit leukotriene synthesis or block symptoms when aspirin-sensitive individuals were challenged with higher doses of aspirin (Pauls et al. 2000).

Further studies have been done to show if this experimental evidence translates into clinical benefit. In a study of 40 aspirin-sensitive patients zileuton was given in a double-blind, placebo-controlled manner for a period of 6 weeks (Dahlen et al. 1998). Patients on zileuton noted an improvement in their FEV$_1$, reduced need for rescue medication, less nasal stuffiness, less rhinorrhoea, and improved sense of smell. Although, montelukast a leukotriene receptor antagonist only partially protected patients from aspirin challenges (Stevenson et al. 2000), a recent 4-week double-blind, placebo-controlled clinical trial has shown that it improves pulmonary function and quality of life in aspirin-sensitive asthmatics (Dahlen et al. 2002).

1.2.8e Treatment of specific manifestations

An attack of *acute asthma* caused by either inadvertent intake of aspirin/NSAID or as part of a challenge procedure is best treated with 3-4 puffs of a nebulized β-agonist (Stevenson and Simon, 1993). This is repeated every 10-15 minutes with corticosteroids until the attack passes, but rarely it may prove refractory in which case intubation and ventilation may be required. Published guidelines for management of acute asthma should be followed in treating these reactions (The BTS/SIGN guidelines. 2003). For nasal reactions a topical decongestant is useful, and ocular symptoms can be relieved with antihistamines.

Long-term control of *asthma* in aspirin-sensitive patients usually requires topical corticosteroids, but 50% need regular daily oral corticosteroids for effective control (Szczeklik et al. 2000). If only a low dose of oral corticosteroids is needed to achieve control it can be given over a long period. However, if large doses are needed alternative therapies have to be attempted.

Patients with chronic *urticaria* should avoid aspirin/NSAIDs. Dietary measures should be instituted as they tend be more effective in this group of patients. Acute urticarial reactions are treated with oral or injected antihistamines. Occasionally, adrenaline 1:1000 is required for additional control. Also, for chronic urticaria antihistamines and leukotriene receptor antagonists have been shown to be beneficial (Pacor et al. 2001).

1.2.8f Treatment of nasal disease

Polyps, which are a manifestation of nasal disease in aspirin-sensitive patients, are managed in an identical manner to those in aspirin tolerant patients. Topical corticosteroids are prescribed for daily use to contain polyp growth. The beneficial effect has been shown in a double-blind, placebo-controlled, cross-over trial on 15 aspirin-sensitive patients (Mastalerz et al. 1997). Improvement of nasal inspiratory peak flow, symptoms scores, and sense of smell were noted. If nasal blockage is severe and topical corticosteroids are unable to impact upon it, a short course of oral corticosteroids will be needed. Nasal polypectomy is reserved for situations where medical options have failed.

Surgery on polyps fell into disrepute following an observational study, which concluded that in patients with the triad a polypectomy would probably make the asthma worse (Francis, 1929). This has been disputed and shown to be incorrect by more recent studies (Brown et al. 1979; Schenck, 1974). Other studies have shown that there is a beneficial effect of surgery on asthma. In a study of 205 aspirin-sensitive asthmatics 34% of patients who required alternate day oral corticosteroids could discontinue them following surgery (English, 1986). Their follow-up period was 6 months. However, the study also showed that patients with severe asthma requiring high doses of daily oral corticosteroids did not benefit from surgery. Overall, 84% benefited considerably from surgery, 14% showed less improvement, and 2% remained the same. Such high figures of improvement in pulmonary function have also been shown more recently (McFadden et al. 1997; Nakamura et al. 1999).

The type and extent of surgery required in aspirin-sensitive patient has been evaluated. A radical approach was advocated if the maxillary and/or sphenoid sinuses were opaque on radiology (McFadden et al. 1990). The surgery recommended included a Caldwell-Luc procedure wherein the maxillary sinus mucosa was removed via a buccal incision, and a transantral opening of the ethmoid/sphenoid sinuses if they were involved. It was speculated that such a radical removal of diseased membrane would improve the patients' asthma and reduce the need for revision surgery. In another retrospective study of 39 aspirin-sensitive patients who had undergone surgery, comparison was made between a radical and conservative approach (Rosen et al. 1996). After a longer follow-up period (average = 46.3 months), revision surgery rates did not differ between the groups. Since revision surgery was highly likely in this group of patients it was suggested that a conservative approach should always be taken. The proponents of radical surgery have also come to the same conclusion after a study with larger number of patients and longer follow-up (McFadden et al. 1997). In addition, the type and extent of surgery does not seem to influence postoperative lung function (Nakamura et al. 1999).

Studies specifically evaluating antileukotriene drugs in nasal disease have been conducted. Improvement in the sense of smell has been noted after Zileuton (5LO inhibitor) use in patients with asthma and nasal polyps (Gretchen Wooding and Incaudo, 1996). In a study on 40 aspirin-sensitive patients, Montelukast (LT receptor antagonist) was compared to mometasone furoate plus loratadine (Di Rienzo et al.

2000). All patients underwent endoscopic sinus surgery prior to randomisation into the two groups. After a 6-month follow-up, patients in both groups were free of nasal polyps and had good nasal patency. A similar conclusion was drawn from another trial using the same drug i.e. montelukast (Grundmann and Topfner, 2001). In this study on 18 aspirin-sensitive patients' clinical improvement was noted along with anti-inflammatory effects at a cellular level. A reduction in EG_2 positive cells and IL-5 levels was seen in inferior turbinate biopsies. A more recent open study has shown that montelukast results in a subjective improvement of nasal symptoms in approximately 50% of aspirin-sensitive patients as compared to 64% of aspirin tolerant patients (Ragab et al. 2001), but the aspirin-sensitive responders tend to show a greater degree of improvement.

1.2.9 *Desensitization*

To sensitize is 'to make (a person) sensitive' (The Shorter Oxford English Dictionary) (Little et al. 1988). Thus, desensitization would be to make (a person) insensitive. In the context of drugs this concept is not new and was used in cases of antipyrine hypersensitivity. The process involved giving small amounts of the drug to the patient. However, at the time opinion was against desensitization in aspirin-sensitive patients as it was considered unsatisfactory and hazardous (Prickman and Buchstein, 1937).

1.2.9a Refractory period

In aspirin-sensitive individuals there is a time phase after aspirin ingestion wherein repeat intake will not lead to adverse reactions. This has been called the 'refractory period'. Although, its existence was first recognized in 1922 (Widal et al. 1987), the phenomenon was accidentally rediscovered during oral challenges on a patient to diagnose their aspirin-sensitivity (Zeiss and Lockey, 1976). To ascertain the existence of this phenomenon the patient underwent an oral challenge on 5 separate occasions and on each of these there was a clear indication of a refractory period. This period lasted 72 hours. The patient was also challenged with indomethacin, but it provoked a severe reaction and the quest to determine the existence of a refractory period with this NSAID was abandoned. A refractory period has also been found in

patients following bronchial provocation with lysine-aspirin (Bianco et al. 1977), and after nasal challenge (Patriarca et al. 1991b). Additional confirmation about the presence of this period came from other studies. In a study on 2 aspirin-sensitive patients, both could tolerate daily aspirin after an initial positive oral challenge (Stevenson et al. 1980). The dose of aspirin was gradually increased to 1200mg/day in one patient, and 625mg/day in the other without any adverse effects. Temporal features of the refractory period were determined in another study (Pleskow et al. 1982). Its length was established in 16 patients. In the majority it was between 2 and 5 days. A gradual return to sensitivity occurred between 2 to 4 days. Other investigators found that the refractory period ranged from 24 hours to 9 days (Kowalski et al. 1984). An additional observation was absence of an adverse reaction to indomethacin during the refractory period, which resulted from aspirin intake (Kowalski et al. 1984; Pleskow et al. 1982).

1.2.9b Clinical studies on desensitization

i) Observation

During a study on 2 aspirin-sensitive patients to investigate their refractory periods it was noticed that they had a marked improvement in their clinical condition (Stevenson et al. 1980). Within 6 months of daily aspirin use their nasal symptoms had resolved, nasal examination revealed a healthy lining, FEV_1 increased, and oral corticosteroid intake had reduced. This phenomenon was called 'desensitization'. A further study from the same group on 30 aspirin-sensitive patients showed that all could be desensitized by oral aspirin challenge using incremental doses (Pleskow et al. 1982). The dose of aspirin needed for desensitization ranged from 33 mgs to 1083 mgs. Majority of the patients (60%) were desensitized after their initial challenge. It was noted that individuals requiring a small dose to provoke their initial reaction needed many more challenges prior to reaching a desensitized state. In 3 patients cross-desensitization with indomethacin was attempted with success. These studies provided a framework for further work to investigate the potential therapeutic benefits of desensitization.

ii) 'Open' trials

The results of 2 open trials were reported simultaneously in 1983 providing evidence on the beneficial effects of desensitization (Chiu, 1983; Lumry et al. 1983). One of the trials showed that patients could be desensitized rapidly within an average time of 322 minutes (Chiu, 1983). The 12 patients in this trial were maintained on a daily dose of aspirin averaging 539 mgs. Six patients (50%) noticed a symptomatic improvement, 3 (25%) were unchanged, and 3 noticed a deterioration of their asthma. The other trial included 19 aspirin-sensitive patients, exclusively with nasal symptoms (Lumry et al. 1983). Following desensitization 17 patients were maintained on a minimum daily dose of 325 mgs. Thirteen patients (77%) reported symptomatic improvement. This included a reduction in nasal obstruction, rhinorrhoea, and an improvement in the sense of smell. Eight of these 13 patients noticed an initial and continued improvement. The average daily aspirin dose in these 8 patients was 1300 mgs. Two patients experienced initial benefit but with continued intake of aspirin, suffered from a recurrence of their symptoms despite a further increase in their maintenance dose up to 2600 mgs. The remaining 3 could not continue with daily aspirin because of side effects such as gastrointestinal upset and easy bruising.

iii) 'Controlled' trials

The therapeutic benefits of desensitization were examined more rigorously in a randomised, double-blind, placebo-controlled, cross-over trial (Stevenson et al. 1984). Thirty-eight aspirin-sensitive subjects were enrolled of whom 25 (66%) completed both phases. They were desensitized before randomisation into either an active or placebo phase, each lasting 3 months. After an intervening 1-month washout period they entered the opposite phase. The subjects maintained a daily diary of nasal and chest symptom scores, and topical corticosteroid use. They were examined every month and FEV_1 measured. Improvement was noticed by 16 (76%) patients. Of the 12 patients on regular prednisolone, 8 (67%) could reduce their daily dose. However, more patients had noticed an improvement in their nasal symptoms compared to chest symptoms (67% vs. 48%). An important factor in analysis was the different maintenance doses given to the 25 patients. The small number of subjects

receiving each dose made subgroup analysis difficult. However, there was a tendency for greater improvement in patients receiving a higher maintenance dose. Another observation was a tendency for patients to partially 'escape' from the desensitized state towards the end of their 3-month active phase. Only 1 of 9 patients (11%) on a high maintenance dose demonstrated 'escape'. These observations suggested the possibility of a dose response relationship in aspirin desensitization.

The presence of a refractory period after nasal challenge (Patriarca et al. 1991b) prompted a novel study wherein lysine-aspirin (LAS) was used intranasally and its impact on nasal polyp growth measured (Patriarca et al. 1991a). Innovations in the trial included topical use of low dose aspirin (2000μg) and an objective measurement of polyp growth. Forty-three patients with nasal polyps (28 aspirin-sensitive, 15 aspirin tolerant) received topical LAS. A control group of 191 patients with nasal polyps (130 aspirin-sensitive, 61 aspirin tolerant) did not receive any topical treatment. Both groups were followed for 24 months with 3 monthly examinations and measurements. Patients on topical LAS, both aspirin-sensitive and aspirin tolerant, did better than those not on it. Recurrence of nasal polyps was seen in 21% of patients on topical LAS compared to 76% of the control group. Subgroup analysis of aspirin-sensitive patients revealed a recurrence rate of 32% in those who received topical LAS compared to 81% of aspirin-sensitive controls. None of the 15 aspirin tolerant patients on LAS had a recurrence, whereas 67% of the aspirin tolerant controls relapsed. More importantly, no patient with asthma noticed deterioration and there were no systemic side effects.

iv) <u>Studies on long-term oral desensitization</u>

It was evident from the trials discussed above that desensitization benefited patients in the short-term. Some of the same groups conducting the trials continued their patients on daily aspirin to observe the long-term effects. One study followed 107 aspirin-sensitive patients divided into 3 groups; 35 desensitized patients on continuous treatment for a mean duration of 3.75 years, 30 desensitized patients who discontinued treatment after a mean duration of 2 years, and 42 patients who avoided aspirin and served as controls (Sweet et al. 1990). Aspirin-sensitive patients in their desensitized state had significantly less hospitalisations, reduced need for polypectomies, an improvement in the sense of smell, and reduced requirement for

oral corticosteroids or short bursts of the same. A significant reduction in inhaled corticosteroid use was seen only in the group that continued on aspirin. Despite the success of desensitization with oral aspirin a large number of patients (20%) suffered from gastrointestinal side effects.

Another study on 65 aspirin-sensitive patients gave further insight into benefits of long-term desensitization (Stevenson et al. 1996). The patients were subdivided into 2 groups; those followed for 1-3 years (n = 29), and those followed for 3-6 years (n = 36). All the benefits mentioned above were also noted in these 65 patients. Mean oral prednisolone intake fell from 10.5mg to 2.5mg. Mean nasal corticosteroid dose reduced from 139μg to 106μg. Subgroup analysis revealed that patients followed for a longer duration i.e. 3-6 years required significantly less sinus or polyp operations. Again, 9 patients (14%) had to stop treatment due to gastrointestinal side effects.

Recently, the results of a prospective study on 30 aspirin-sensitive patients, given a low dose (100mgs) of aspirin orally, and followed for 1 year has been published (Gosepath et al. 2001). Only 4 patients had evidence of polyp recurrence, and 9 patients (of 12) with asthma noticed a marked improvement in lung function. Other noticeable improvements were in nasal airway and sense of smell.

v) <u>Studies on long-term intranasal desensitization</u>

Two studies advocating the use of intranasal LAS in patients with nasal polyposis and follow-up periods of 3 and 6 years have been reported recently (Nucera et al. 2000). Patients with aspirin-sensitive and aspirin tolerant nasal polyps were included in both studies. For both studies 191 patients who did not have any medical treatment post-operatively served as controls.

The first was a 6-year follow-up study on 76 polyp patients treated with surgery, and 1-month post surgery started on intranasal LAS 4mg, which was instilled 6 times/week. Cumulative recurrence rates of polyps compared to controls were as follows: after 1 year 6.9% vs. 51.3%, after 3 years 44.9% vs. 84.8%, and after 6 years 65% vs.93.5%. Although, topical LAS worked for all nasal polyp patients, no differences could be elicited between aspirin-sensitive and aspirin tolerant patients within the group or compared to controls.

The second was a 3-year follow-up study on 49 polyp patients who underwent a medical polypectomy and were subsequently started on intranasal LAS as described

above. Cumulative percentages of patients requiring surgical intervention were: 3.8% at 6 months, 13.1% at 1 year, and 32% after 3 years. Figures for the control group are 51.3%, and 85% at year 1 and 3 respectively. Once again, no differences could be shown between aspirin-sensitive and aspirin tolerant patients.

1.2.9c Mechanism of desensitization

Following initial studies evaluating the effects of desensitization several hypotheses were proposed for its mechanism (Pleskow et al. 1982; Stevenson et al. 1980). An immunologic theory was ruled out in favour of a biochemical one as cross desensitization between aspirin and indomethacin had been shown.

Circumstantial evidence suggested a role for mast cells. In 7 aspirin-sensitive asthmatics plasma histamine levels increased following oral aspirin challenge as opposed to no change in aspirin tolerant asthmatics (Stevenson et al. 1976). This rise coincided with the onset of the adverse reaction and was highest in patients with the most severe reaction. Thus, it was proposed that aspirin acted via receptors on mast cell surface leading to degranulation and release of mediators and subsequent reaction. If aspirin intake continued mast cell mediator stores would be depleted, leading to a state of desensitization. If aspirin intake was halted, mast cells could revitalise their mediator stores. This would also explain the temporal features of an aspirin-induced refractory period. Indirect activation of mast cells via the complement system was another suggestion (Stevenson et al. 1976). This was not a new idea and had already been discussed as a possible explanation of aspirin-sensitivity (Yurchak et al. 1970). It was known that certain substances like dextran or endotoxin could directly activate the complement system. Formation of $C3_a$ and its subsequent action on mast cells could lead to histamine release. Prevention of an aspirin-induced reaction following pre-treatment with antihistamines (Szczeklik et al. 1980), or cromolyn sodium (Basomba et al. 1976), provided further evidence that mast cell release of histamine played an important role.

As leukotrienes became central to our understanding of aspirin-sensitivity their role in desensitization was investigated. It was shown that 1 day after oral aspirin desensitization the responsiveness of the airways to LTE_4 reduced 20-fold (Arm et al. 1989). Such an effect was not seen in aspirin tolerant patients. It was suggested that this might be due to desensitization of the LTE_4 receptor following persistent

stimulation by constant mediator release secondary to repeated aspirin intake. This possibility was revisited when a subsequent study demonstrated a rise in baseline urinary LTE_4 levels after chronic oral aspirin desensitization (Nasser et al. 1995). Further research showed that aspirin desensitization was accompanied by a reduction in leukotriene production (Juergens et al. 1995; Nasser et al. 1995). The rise in urinary LTE_4 levels accompanying an acute reaction reduced 4-fold after chronic aspirin desensitization (9 ± 3 months) with a daily dose of 600mgs (Nasser et al. 1995). In another study, release of leukotrienes from monocytes of 10 aspirin-sensitive subjects reduced significantly after aspirin desensitization (LTB_4; 861±139 pg/ml to 484±85 pg/ml) (Juergens et al. 1995).

AIMS

2.1 *Primary aim*

To undertake a clinical trial testing the hypothesis that in *aspirin-sensitive* patients with nasal polyps, regular long-term low-dose intranasal aspirin is more effective in reducing polyp growth rate compared to a placebo.

2.2 *Secondary aims*

2.2.1

To undertake a clinical trial testing the hypothesis that in *aspirin-tolerant* patients with nasal polyps, a combination of regular long-term low-dose intranasal aspirin and topical corticosteroid is more effective in reducing polyp growth rate compared to a combination of placebo and topical corticosteroid.

2.2.2

To study tissue from *aspirin-sensitive* patients and advance our understanding of:

i. Pathogenesis of aspirin-sensitivity
ii. Mechanism of desensitization

PLAN OF INVESTIGATION

To fulfil the aims outlined in the previous section we planned several studies. In this chapter I discuss their protocols. These studies were not sequential but proceeded simultaneously. Details of various methods employed are in the next chapter.

3.1 Characterization of patients

3.1.1 *Objective*

To determine if a patient was aspirin sensitive or tolerant, and to build a database of these two groups. This was an essential step prior to enrolling patients in the appropriate trials.

3.1.2 *Design*

Patients with nasal polyps, seen in the specialist Rhinology clinic at the Royal National Throat, Nose and Ear Hospital (RNTNEH), were invited to have their aspirin-sensitive status determined. This was done by undertaking a single-blind, placebo-controlled, intranasal lysine-aspirin challenge. It was conducted under controlled circumstances in the Rhinology laboratory situated on the premises of the RNTNEH.

3.1.3 *Outcome measures*

A reduction in nasal volume of 25%, with or without a 25% decrease in minimum cross-sectional area was taken as the main indicator of a positive challenge. Other parameters measured were symptom scores, peak flow rate, and dynamic nasal airflow.

3.2 Trial 1

3.2.1 *Objective*

To study the clinical effectiveness of intranasal lysine-aspirin, in reducing nasal polyp growth, in *aspirin-sensitive* patients with nasal polyposis.

3.2.2 *Design (Figure 3.1)*

A randomised, double-blind, placebo-controlled, crossover trial. Aspirin-sensitive patients were invited to take part in this trial. An information sheet providing details of the trial was given to interested patients along with a consent form (Appendix A, B). If a patient agreed to take part they were requested to attend the laboratory at a mutually convenient time. The consent form was collected and further queries were addressed.

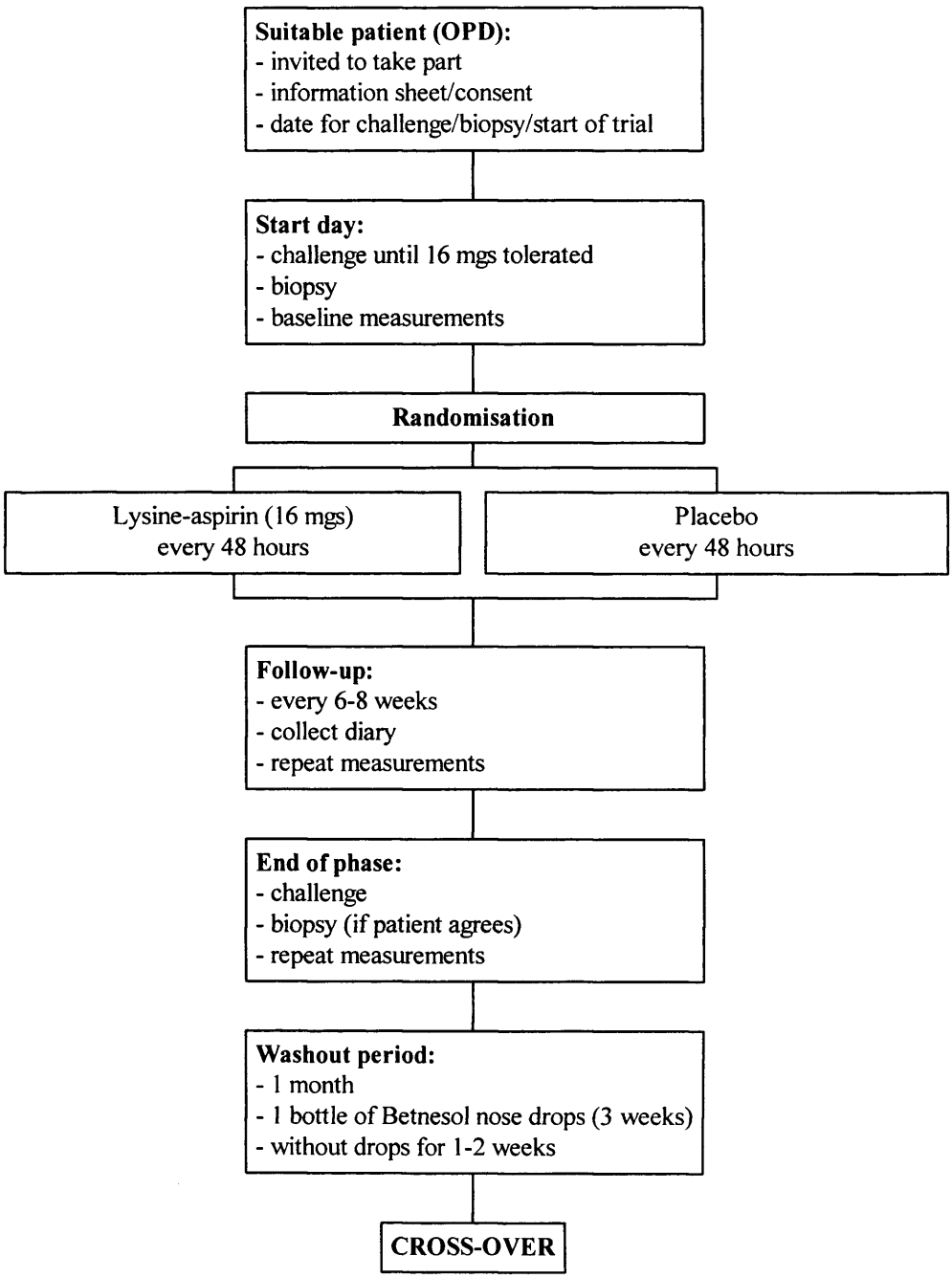
Once enrolled the patient underwent repeated intranasal challenges with incremental doses of lysine-aspirin until they tolerated a dose of 16 mgs. Then they were randomised to receive either placebo or lysine-aspirin. Drops were used intranasally every 48 hours, considering the baseline visit as day 1. Instructions for preparing and correctly instilling the drops were provided (Appendix C).

Subjects remained in their respective phases for a period of 6 months or until their nasal symptoms deteriorated, whichever came first. Apart from the trial medication no other intranasal or oral drugs were allowed that would impact on their nasal inflammation. During this period they were reviewed every 6 weeks in the laboratory for clinical assessment and measurements.

At the last visit the subjects were re-challenged as at first visit with incremental doses of lysine-aspirin up to 16 mgs. In addition, they had clinical assessment and measurements. Following this visit the patients entered a 1-month 'washout' period. During the washout period the patients were instructed to use Betamethasone sodium phosphate 1% nose drops (Betnesol®), 2 drops in each nasal cavity twice daily. If the drops finished earlier than a month they were asked to use an intranasal corticosteroid of their choice until 2 weeks before their baseline visit for the next phase of the trial. This baseline visit was identical to the patients initial enrolment

Figure 3.1

Profile of Trial 1



Measurements at baseline and each visit:

- Clinical evaluation
- Peak expiratory flow rate
- Nasal inspiratory flow rate
- Rigid nasendoscopy
- Acoustic rhinometry

visit, the only difference being that they were 'crossed over' by the pharmacy as regards the medication.

3.2.3 *Inclusion criteria*

i. Patients above 18 years
ii. Nasal polyps on clinical examination
iii. History of aspirin-sensitivity confirmed by a positive intranasal lysine-aspirin challenge

3.2.4 *Exclusion criteria*

i. Patients with poorly controlled asthma requiring regular or intermittent oral corticosteroids
ii. Pregnant or lactating females
iii. Patients with a severe deviation of the nasal septum

3.2.5 *Withdrawal or drop-out from the trial*

Patients were withdrawn from a phase of the trial if their symptoms deteriorated and adversely affected their quality of life. They were offered an 'emergency' visit, which was considered as the last visit for that particular phase. The protocol as for any last visit was followed. These patients were entered into the washout phase and treated with betamethasone sodium phosphate 1% nose drops. After this period, which lasted 4-6 weeks they were crossed over. Patients with a poor response to the drops were offered either a medical (Drake-Lee, 1994), (Mygind and Lindholt, 1996) or surgical polypectomy. In these cases we waited approximately 8 weeks prior to crossover.

Patients who did not attend scheduled follow-up appointments, and stopped using their intranasal medication, were contacted by telephone for an explanation. If they were still using their medication but wanted to drop-out, we suggested a final visit to the laboratory for challenge, clinical assessment, and measurements.

3.2.6 *Randomisation*

The pharmacy personnel at RNTNEH generated the randomisation code. This allocation schedule was held within the pharmacy. None of the patients, clinicians, or ancillary staff involved in the trial were privy to the allocation schedule. The code was concealed until preliminary analysis of the data was completed.

3.2.7 *Medication*

Capsules of lysine-aspirin and placebo (lysine), along with their diluents (normal saline), were prepared and provided by Laboratories for Applied Biology under the guidance of its Medical Director, Dr. A. Freedman MD, FRCP.

The colour of the capsules, the powder within them, and diluent for both placebo and lysine-aspirin were colourless. The prepared solutions did not have a distinct taste and hence could not be distinguished. Capsules had a shelf life of 3 months when stored in a cool, dry place. These instructions were given to the patient. The pharmacy personnel maintained a stock of capsules, diluent, and received all deliveries. This gave an added degree of control on blinding.

3.2.8 *Calculation of sample size*

To help us in our calculations of an adequate sample size we utilized the results from a published study in which intranasal lysine-aspirin was used to desensitize patients with nasal polyps (Patriarca et al. 1991a).

The sample size was calculated using the following formula (Florey, 1993):

i. $n = (\alpha + \beta)^2 (p_1q_1 + p_2q_2) / \Delta^2$, where

ii. n = sample size

iii. p_1 = % of patients responding to active treatment (68%)

iv. p_2 = % of patients responding to placebo (18%)

v. q_1 = % of patients not responding to active treatment (32%)

vi. q_2 = % of patients not responding to placebo (82%)

vii. Δ = Effect size we would like to see (50%)

viii. α = probability of a type I error (5%)

ix. β = probability of a type II error

Using the results from the published study (Patriarca et al. 1991a), the values for these factors are $p_1 = 68\%$, $p_2 = 18\%$, $q_1 = 32\%$, $q_2 = 82\%$, $\Delta = 50\%$. The multiplier for the conventional values of α (5%) is 1.96, and that for β is 0.842. Thus, n = (7.85) (2176 + 1476) / 2500 = 11.46 = 12 patients. Taking into consideration withdrawals and dropouts we concluded that 20 patients would be an adequate sample size.

3.2.9 *Outcome measures*

i. Primary

- To compare nasal polyp growth rate in *a patient* while on intranasal lysine-aspirin with the rate when on placebo.
- To compare nasal polyp growth rate of *patients* on intranasal lysine-aspirin with those on placebo.

ii. Secondary

- To monitor the peak flow rate in patients on intranasal lysine-aspirin and note any deterioration in asthma control.

3.3 Trial 2

3.3.1 *Objective*

To study the combined efficacy of intranasal lysine-aspirin and intranasal corticosteroid, in reducing nasal polyp growth, in *aspirin-tolerant* patients with nasal polyposis.

3.3.2 *Design (Figure 3.2)*

A randomised, double-blind, placebo-controlled, parallel group trial, in aspirin-tolerant patients. Suitable patients were given an information sheet providing details of the trial along with a consent form (Appendix F, G). If a patient agreed to take part, baseline measurements were done in the laboratory following which he/she was randomised to receive either placebo or lysine-aspirin. Patients prepared a solution at home and sprayed the equivalent of 16 mgs, once a week. In addition, all patients were given an intranasal corticosteroid for daily use. Follow-up visits were arranged every 3 months for clinical assessment and measurements.

3.3.3 *Inclusion criteria*

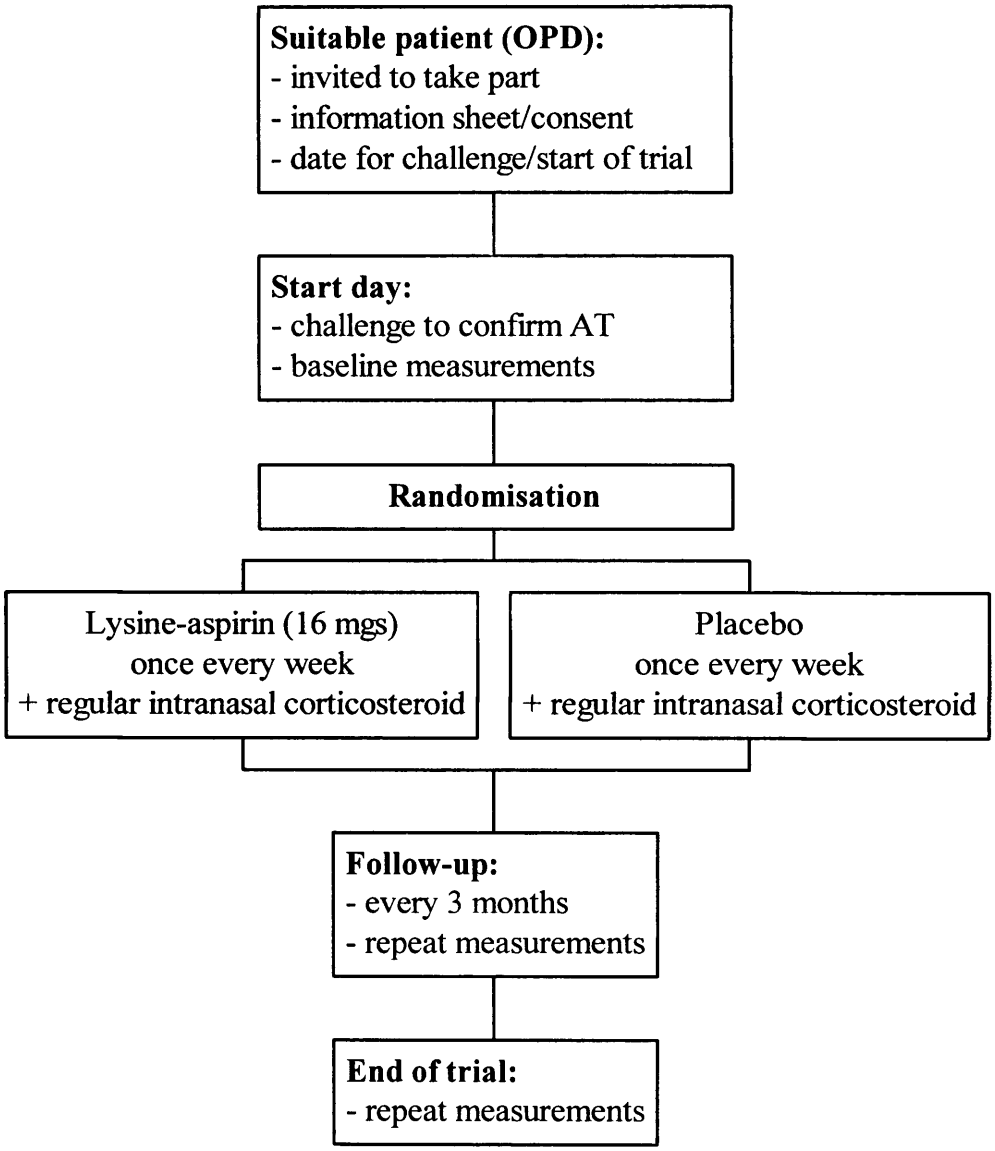
i. Patients above 18 years
ii. Nasal polyps on clinical examination
iii. History of aspirin tolerance confirmed by a negative intranasal lysine-aspirin challenge

3.3.4 *Exclusion criteria*

i. Pregnant or lactating females
ii. Patients with a severe deviation of nasal septum

Figure 3.2

Profile of Trial 2



Measurements at baseline and each visit:

- Visual analogue scale of nasal symptoms
- Peak expiratory flow rate
- Nasal inspiratory flow rate
- UPSIT (smell test)
- Rigid nasendoscopy (appearance, grade of polyps)
- Acoustic rhinometry
- Quality of life questionnaire

3.3.5 *Withdrawal or dropout from the trial, Randomisation, Medication*

These aspects of the trial were identical to Trial 1.

3.3.6 *Calculation of sample size*

The same formula as in Trial 1 was used and the results from a preliminary study (Scadding et al. 1995) were utilized to derive some values. Thus, for this trial p_1 = 40%, p_2 = 15%, q_1 = 60%, and q_2 = 85%. The effect size we considered was Δ = 50%. Values of α , and β were identical to Trial 1.
Thus, n = (7.85) (2400 + 1275) / 5625 = 40.25 = 12 patients in each group.

3.3.7 *Outcome measures*

i. Primary
   - To compare nasal polyp growth rate of patients on intranasal lysine-aspirin with those on placebo.

ii. Secondary
   - To study the quality of life in patients with nasal polyps over a long term.
   - To study the effect of intranasal lysine-aspirin on the patients' sense of smell as compared to placebo.

3.4 Laboratory based studies

3.4.1 Immunohistochemistry of nasal biopsies

3.4.1a *Objective*

To measure expression of the $CysLT_1$ receptor on inflammatory leukocytes in nasal biopsies from aspirin sensitive and non-aspirin sensitive subjects with rhinosinusitis. To compare the expression of the $CysLT_1$ receptor with that of B-LT receptor, in aspirin-sensitive subjects, after 6 months of desensitization with topical lysine aspirin or placebo control.

3.4.1b *Design*

Patients participating in Trial 1 were requested to have a biopsy from their nasal mucosa. Biopsies were taken whenever possible at baseline, and at the end of each phase. The specimens were snap frozen and stored in liquid nitrogen. Immunohistochemical analysis was done after all partaking patients had completed the trial.

Whilst undertaking the clinical trials we started a collaborative project with another research group primarily interested in elucidating the mechanism of aspirin-sensitivity. Clinical information and tissue samples were provided by us and laboratory based analysis was undertaken by the reciprocating group.

3.4.2 Expression of inducible nitric oxide synthase (iNOS)

3.4.2a *Objective*

Inducible nitric oxide synthase (iNOS) expression is upregulated in nasal polyp epithelium implying a role for nitric oxide (NO) in their formation (Watkins et al. 1998). We decided to compare iNOS activity in polyp tissue from patients with and without aspirin-sensitivity.

3.4.2b *Design*

Nasal polyp tissue was collected from patients undergoing routine nasal polypectomy. It was stored in liquid nitrogen until the start of analysis. Patients were divided into 3 groups - Group A were aspirin-tolerant patients with nasal polyps without asthma; Group B were aspirin-tolerant patients with nasal polyps and asthma; Group C were aspirin-sensitive patients with nasal polyps and asthma. Group C patients had a history of aspirin-induced reaction and a confirmatory intranasal challenge with lysine-aspirin. NOS activity was measured by the ability of tissue homogenates to convert 3,4 L-arginine to L-citrulline in an L-NAME inhibitable fashion.

METHODS

4.1 Nasal challenge

4.1.1 *Challenge procedure*

Patients with nasal polyps were recruited from the outpatient clinics at RNTNEH. Those willing to undergo an intranasal lysine-aspirin challenge were explained the procedure and possible discomfort they may experience. A suitable appointment was arranged for them to have the challenge in the Rhinology laboratory bearing in mind that in all it took approximately 2 hours.

Patients were requested to stop using intranasal corticosteroids 2 weeks prior to challenge and avoid oral antihistamines for at least 48 hours. Patients with asthma continued using their inhalers as before. Only those patients with well-controlled asthma (peak expiratory flow rate $\geq$ 80%), not requiring oral corticosteroids were invited for the challenge.

On arrival to the laboratory for their challenge the patients spent 15 minutes acclimatizing to the room temperature. Following acclimatization they had a baseline clinical examination, marking of symptoms on a visual analogue scale (VAS), and measurements which included peak expiratory flow rate (PEFR), nasal inspiratory flow (NIPF), and acoustic rhinometry.

The challenge procedure was conducted in a single-blind, placebo-controlled manner. Patients were told that they would be challenged with various doses of lysine-aspirin. They were unaware of the order in which we performed the challenge. Solutions were prepared either prior to the patients' arrival or hidden from their eyesight. A 1 ml syringe was used as a dropper for the solution, which was instilled into the patient's nose with them in the head forward and down position for good distribution. They remained in this position for at least 45 seconds to 1 minute. The receptacles used for preparing solutions of varying strengths were thoroughly rinsed after the day's tests to avoid contamination when preparing fresh solutions another day.

The challenge procedure started with a placebo followed by intranasal lysine-aspirin in incremental doses. These started with a dose of 4 mgs, followed by 8 mgs, and lastly 16 mgs. The interval period between each dose was 20 minutes. If there were

early indications of a reaction we waited an additional period of 20 minutes prior to giving a further, higher dose. Patients stayed within the laboratory throughout the procedure and refrained from hot or cold beverages or foodstuff.

Twenty minutes following each dose measurements were repeated. These were similar to those done at baseline apart from clinical examination. At the end of the procedure patients were informed of the results. Patients with a positive challenge were observed for a further 40-60 minutes to ensure a good peak flow rate. Aspirin-sensitive and tolerant patients were also informed about our on-going trials, and invited to participate.

4.1.2 *Criteria for positive challenge*

The parameters considered significant were:

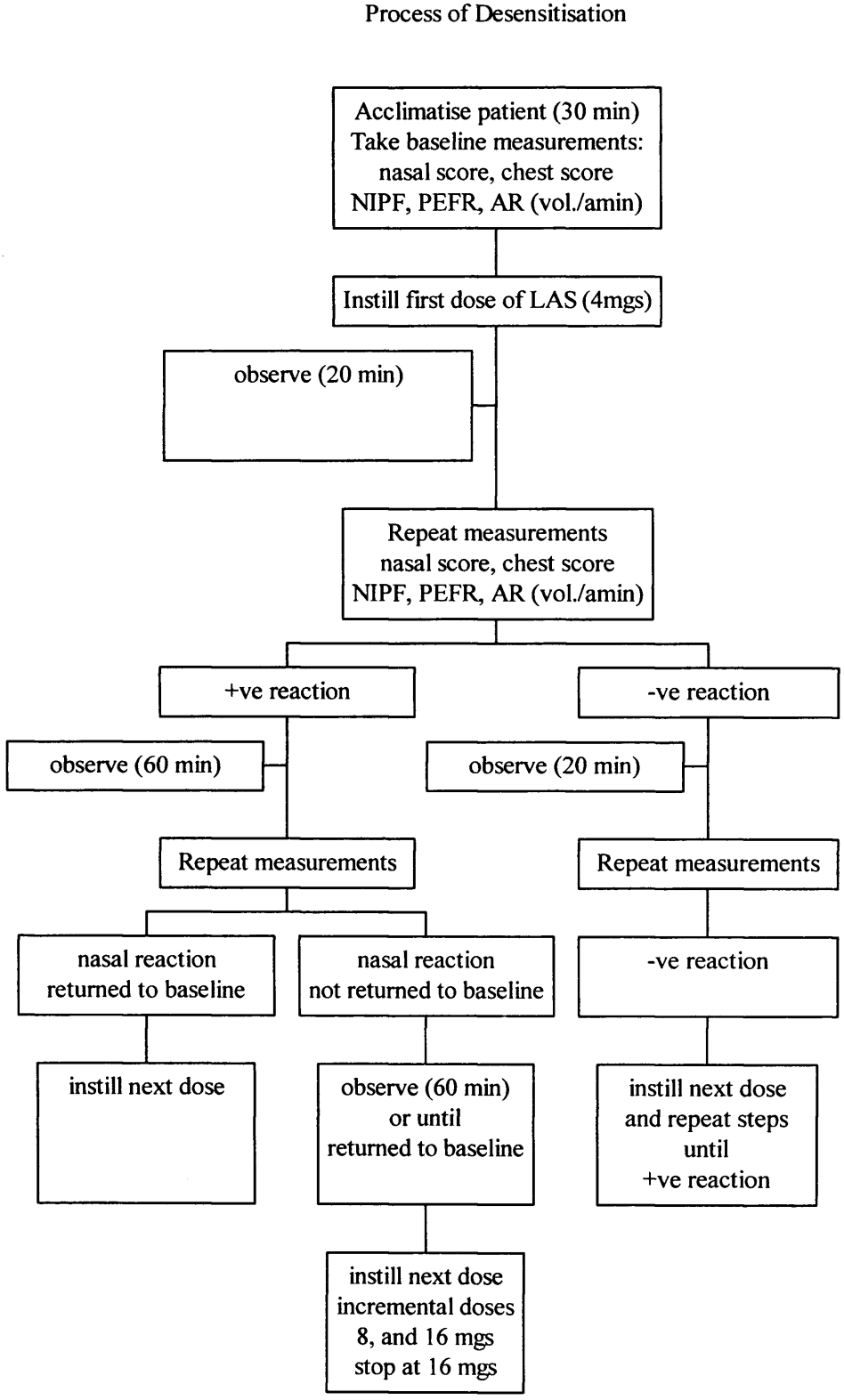
i. A 25% reduction in nasal volume measurements on acoustic rhinometry (AR).
ii. A 25% reduction in minimum cross-sectional area of the nose on AR.
iii. A 25% reduction in nasal inspiratory flow (NIPF).
iv. A 25% fall in peak expiratory flow rate (PEFR) from baseline.
v. A 10-point increase in composite visual analogue scale (VAS) scores.

The percentage changes for the measurements were derived after considering the variability of each parameter. The challenge was considered positive if 2 out of 5 parameters were present.

4.1.3 *Method of desensitisation* (Figure 4.1)

Aspirin-sensitive patients who agreed to take part in our trial of desensitisation underwent this process immediately after their diagnostic nasal challenge or at a later date depending on their availability. Topical desensitisation was achieved by its intranasal application in incremental doses, starting with 4 mgs, followed by 8 mgs, and finally 16 mgs. Lysine-aspirin solution of different strengths were prepared freshly on the day, and kept in separate labeled containers. The correct amount was drawn up in a 1 ml syringe, and instilled in the nose with the head in a down and forward position. The first dose was instilled following 30-minute of acclimatisation

Figure 4.1



Key: NIPF: nasal inspiratory peak flow; PEFR: peak expiratory flow rate; AR: acoustic rhinometry; vol.: volume (0-7 cms); amin: minimal cross-sectional area; LAS: lysine-aspirin; min: minutes; mgs: milligrams

period. Reaction was monitored by recording nasal score, chest score, nasal inspiratory peak flow, peak expiratory flow rate, and acoustic rhinometry. Measurements were made at baseline, and subsequently at 20-minute intervals. The next incremental dose was instilled if after 2 observation periods (40 minutes) elapsed without a reaction. In patients with a reaction the interval to the next incremental dose was 1 hour. If the acoustic rhinometry measurements had not returned close to baseline, we waited a further 1-hour. Desensitisation was stopped once we reached our top dose of 16 mgs.

4.2 Clinical assessment

A patient was considered suitable for any of our investigations following an initial clinical assessment in our outpatient clinics. This included history taking, examination with a headlight, and a rigid nasendoscope. The latter confirmed the presence of nasal polyps.

4.2.1 *History*

In history particular emphasis was placed on the presence of asthma, drugs required for its management, and the current state of its control. A history of aspirin-sensitivity or tolerance was determined after direct questioning. Patients were asked if they recall taking aspirin or other NSAID. Names of commonly ingested NSAIDs were directly mentioned. They were asked about any adverse reactions following ingestion. Inquiry included a time course, type of reaction, relief measures, need to attend hospital, and any other relevant factors the patient could remember. If a patient thought he/she was aspirin tolerant, further questions were asked to ascertain their status. They were asked to recall the events following the last time they took aspirin or a NSAID (again, names were mentioned). If they had taken the drug within the past 1 year and could recall not having a reaction, they were considered as tolerant on history.

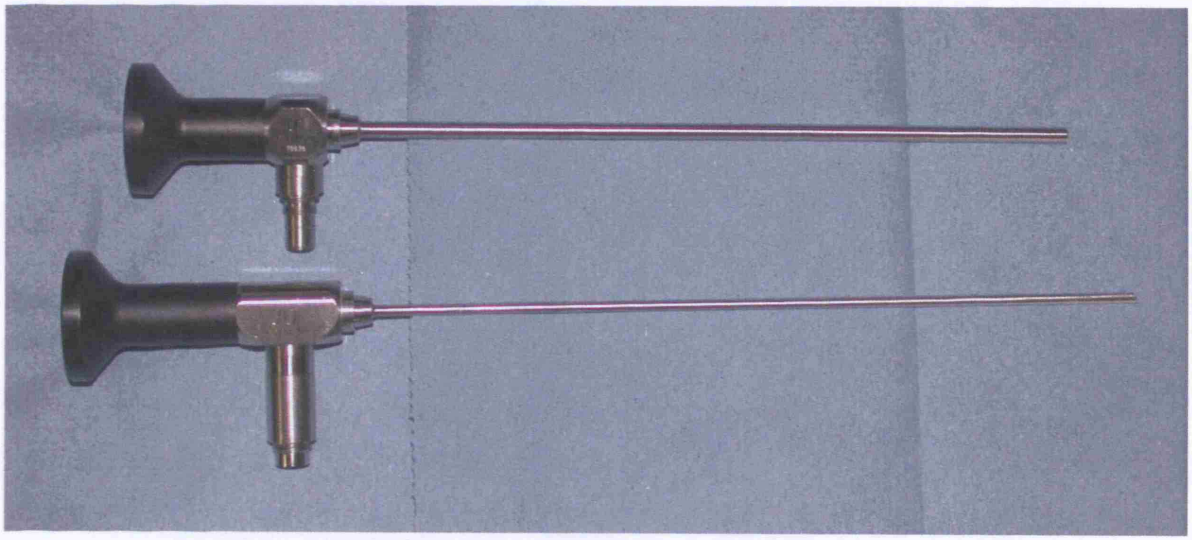
4.2.2 *Rigid nasendoscopy*

The modern rigid nasendoscope consists of a rod-lens system encased in a metal telescope tube. The diameter of a telescope can be 2.7 mm or 4 mm (Figure 4.1). Likewise, the tips can be either flat (0^0) or angled (30^0). These nasendoscopes are illuminated using a cold light source and a fibreoptic cable. The appreciation of intranasal structures is superior to conventional methods of nasal examination. Nowadays this method of nasal examination is considered mandatory in assessing ENT patients, in particular those with nasal complaints.

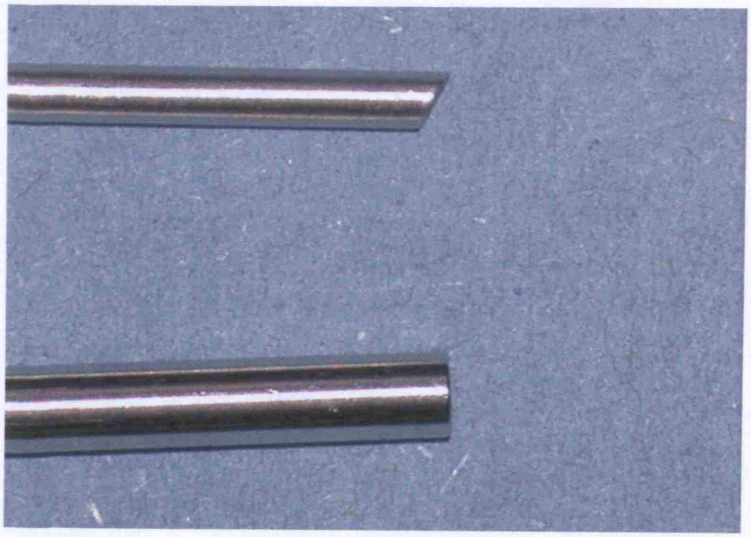
Rigid nasendoscopy was used to examine the patients in the outpatient clinic and confirm the presence of nasal polyps. In both clinical trials (1 and 2), rigid nasendoscopy was used to record polyp growth using a validated staging system (Lund and Mackay, 1993). This system graded polyps from I to III. Polyps confined to the middle meatus were classified as grade I, those beyond the middle meatus but not completely obstructing the nasal cavity were grade II, and those causing total obstruction were grade III. In addition, the presence of other features like congestion, discharge, and crusting were noted, and the appearances were scored using a recommended quantification system (Lund and Kennedy, 1997). This system had 0, 1, or 2 points for these appearances. A score of 0 signified absence of the feature, 1 indicated a mild presence, and 2 meant marked presence. Following nasendoscopy the grade of polyps on both sides, and scores for other features were recorded in the notes or a trial booklet (Appendix H).

Figure 4.2

4 mm and 2.7 mm rigid nasendoscopes



Close up showing an angled tip (30^0), and a flat tip (0^0)



4.3 Visual analogue scale

A validated visual analogue scale (VAS) was used for assessment of symptoms (Lund et al. 1991). It was used during intranasal challenges and during follow-up of patients in trial 2.

The scale has unmarked lines of equal length for each nasal symptom (Appendix E). For nasal challenge the symptoms included sneezing, itching, obstruction, and rhinorrhoea. The left end of the unmarked line was 0, which signified absence of the symptom whereas the right end was 10 signifying symptom of a severe degree. Depending on how they felt, patients were instructed to mark on this line. The relative length from 0 was measured taking the whole length of the line to be 10. After measuring the lines for each symptom the numbers were added to arrive at a composite score, which was used for analysis. An increase in the score indicated a deterioration of nasal symptoms.

The scale was modified for trial 2 (Appendix I). Instead of an unmarked line we used a numbered scale from 0 to 10. Additional symptoms on the scale included headaches, facial pain, and sense of smell. The scale was more explicit making it easier to use. The numbers were added to make a total for each visit. Like the scale for nasal challenge an increase in score indicated deterioration.

4.4 Nasal inspiratory peak flow

4.4.1 *Background*

To study dynamic nasal airflow we measured nasal inspiratory peak flow (NIPF) using a Youlten's meter (Clement Clark International Ltd., London, UK) (Holmstrom et al. 1990). This objective method of assessing nasal patency is cheap, quick, and easy to use. It correlates highly with the total nasal resistance measured by active anterior rhinomanometry (Jones et al. 1991). NIPF has a sensitivity of 80% (confidence interval: CI = 69-100), in detecting changes to nasal patency (Porter et al. 1996).

4.4.2 *Method of use*

The meter has a nozzle to which is attached a cushioned face mask (Figure 4.2). This mask covers the nose and the soft cushion prevents any distortion of nasal contours, which could compromise readings. The patient is asked to empty the lungs and hold their breath. The meter is then applied around the nose using adequate pressure so as to achieve an airtight seal and avoid distorting the contours. The patient is asked to sniff air through the nose without opening their mouth and with a maximally forceful inspiratory effort. The reading on the meter is noted. Measurements are repeated 3 times and the highest value is taken for analysis. The unit of measurement is litres/minute (l/min).

4.4.3 *Measurements*

NIPF was used for measuring changes in nasal patency for all 3 clinical arms of the investigation. It was used to monitor changes during intranasal challenges with lysine-aspirin, which were done to characterize patients. NIPF measurements are reproducible in an individual with a coefficient of variation being approximately 10% (Holmstrom et al. 1990). However, to be definite we chose a figure of 25% to indicate a significant change.

In Trial 1 NIPF measurements were done at every visit to the laboratory. In addition, each patient was provided with a meter to be kept at home. This was used to measure daily NIPF, morning and evening, in between visits. The highest reading was noted in a diary, which was submitted to us at the following visit. In Trial 2 NIPF measurements were made at the follow-up visits every 3 months.

Figure 4.3

Youlten's nasal inspiratory peak flow meter

4.5 Acoustic Rhinometry

4.5.1 *Background*

To detect small changes in the nasal airway we used an objective method to measure its geometrical dimensions. This method is called acoustic rhinometry (Elbrond et al. 1991b). It is based on the principles of 'time domain reflectometry' (Fisher, 1997). Sound waves are generated by a spark and carried up a wave tube into the nose via a Perspex nosepiece (Figure 4.3). These waves are reflected as they encounter various structures within the nasal cavity. The reflected sound waves are detected by a microphone, attached to the wave tube, and analysed by computer software. It generates a graphic representation plotted as an 'area-distance function' showing the cross-sectional area of the airway on the *y*-axis and distance on the *x*-axis (Figure 4.4). The software can calculate the minimum cross-sectional area of the airway between two points *a* and *b*, also called Amin (*a-b*). Integration of the areas under the curve produces a volume estimate denoted by a short form, Vol. Again, the software can generate values for it between two points, Vol (*a-b*).

Acoustic rhinometry is superior to another method of objective nasal patency assessment called anterior rhinomanometry. This is particularly true in assessing nasal challenge because a reaction causes marked reduction in nasal patency, and anterior rhinomanometry may be difficult to perform on an obstructed nose (Scadding et al. 1994). The sensitivity of acoustic rhinometry to detect changes in nasal patency is high; 90% (CI = 76-100) (Porter et al. 1996). Also, acoustic rhinometry has been validated for use in monitoring treatment of nasal polyps (Elbrond et al. 1991; Lindholt, 1989; O'Flynn, 1993).

4.5.2 *Method of use*

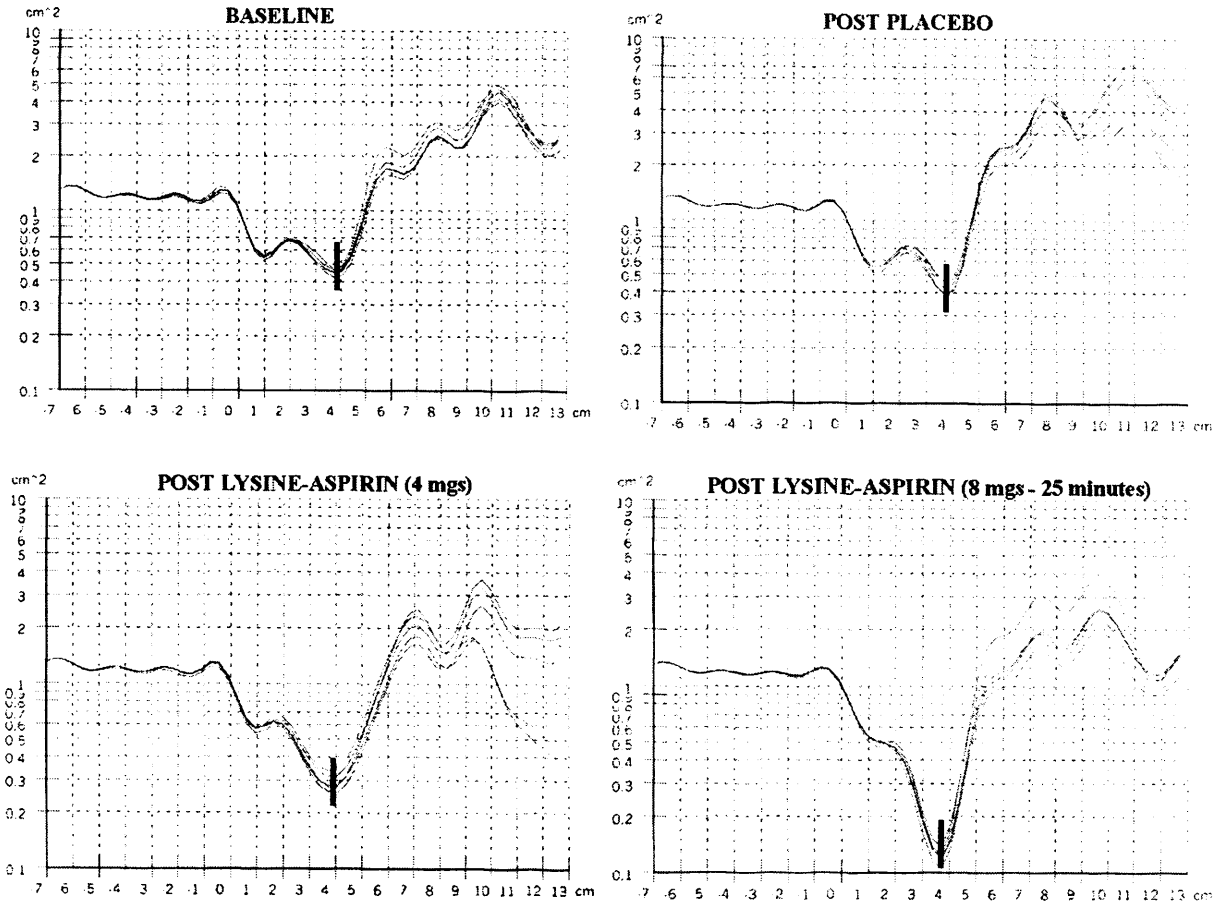
To increase accuracy of measurement and reduce variability certain technical details were observed (Fisher, 1997). We used the same *gm* instruments acoustic rhinometer (Kilwinning, UK) for all measurements. A standardized protocol was used for all patients. Prior to the procedure patients were seated in the laboratory for 15 minutes for acclimatization.

Figure 4.4

Acoustic rhinometry equipment

Figure 4.5

Acoustic Rhinometry output of a positive intranasal lysine-aspirin challenge



The solid line shows changes in $Amin_1$ during a positive nasal challenge:

- Baseline = .45 mm^2
- Post placebo = .40 mm^2
- Post lysine-aspirin (4 mgs) = .28 mm^2
- Post lysine-aspirin (8 mgs – 25 minutes) = .14 mm^2

For the test patients were seated at the same height and a nosepiece that gave a good seal without distortion of the nostril was used. A protractor fixed to the rhinometer box ensured that the wave tube angle was constant and between 35^0-70^0. Patients were asked to hold their breath during the test. To ensure constancy of readings in each patient we used a nosepiece of the same size, and the same angle of wave tube at every visit.

Click sounds separated by 2 milliseconds were used to acquire 5 readings. Inter-reading variability was kept below 10%. The curves generated were printed with patient details and date of test. These were filed as hard copies for future analysis. Also, each curve could be saved on the computer as a single file. Files were named with patient initials followed by date of the test. At a later date these files were recalled into a special program (Acoustic Reports), meant for analysis of the data (Figure 4.5).

4.5.3 *Measurements*

We measured the minimal cross-sectional area (Amin), and Volume (Vol). For nasal challenge, Trial 1, and Trial 2 the volume measurements were between 0-7 centimeters; 0 being at the end of the nosepiece. This distance was chosen as it would include the middle meatus region from where the polyps arise. The sensitivity of volume 0-7 centimeters measurements is 90% (CI = 76-100) (Porter et al. 1996). The curve generated following a test has notches, which signify narrow areas of the nasal cavity. Two such areas have been given names, the 'I' notch and 'C' notch (Lenders and Pirsig, 1990). 'I' indicates isthmus and 'C' is the Amin at the head of the inferior turbinate. We measured, and called the 'C' notch area $Amin_1$. We also analysed the curve and looked for an additional notch deeper into the nasal cavity in the region of the middle meatus from where the polyps grow. We also measured this notch and called it $Amin_2$.

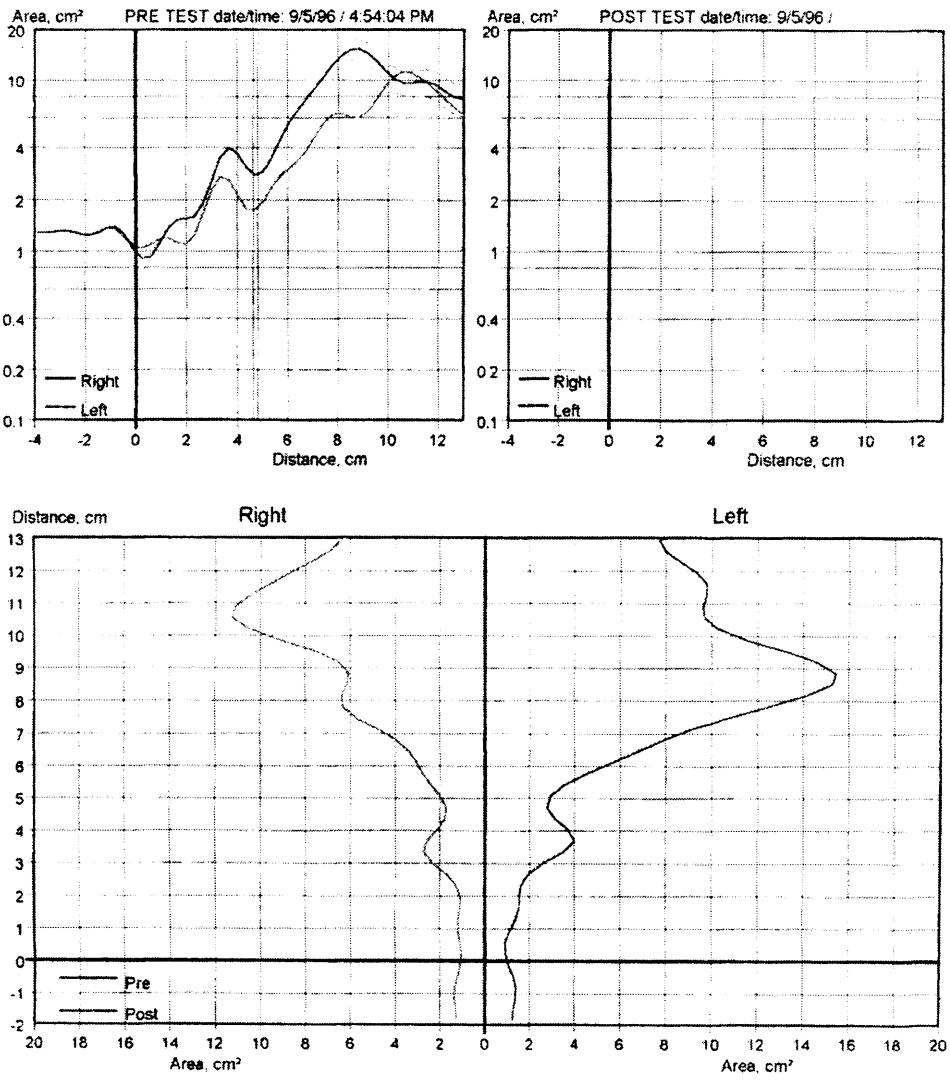
Measurements were made before and after nasal challenge with lysine-aspirin, and at every visit in Trials 1 and 2. For the purpose of analysing nasal challenge, and Trial 2 results we used Vol (0-7) along with $Amin_1$ and $Amin_2$. In Trial 1 we used Vol (0-7) and only $Amin_2$.

Figure 4.6

Output from Acoustic reports programme

Patient: Roddy No: Tested by:

Process:-



		Volume, cm³				Area, cm²				Min area, cm²			
Distance, cm		0 - 7	2 - 4	0 - 5	7 - 13	3.3	4	6.4	13	Area1	(dist)	Area2	(dist)
Pre	Right	14.44	4.03	8.11	46.63	2.71	2.14	3.35	6.34	1.75	(4.6)	0.00	(0.0)
	Left	22.35	5.48	10.83	65.92	3.43	3.72	6.68	7.76	2.81	(4.8)	0.00	(0.0)
	R/L	0.65	0.74	0.75	0.71	0.79	0.56	0.50	0.82	0.62		0.00	
Post	Right	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	(0.0)	0.00	(0.0)
	Left	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	(0.0)	0.00	(0.0)
	R/L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	
Percent change	Right	0	0	0	0	0	0	0	0	0		0	
	Left	0	0	0	0	0	0	0	0	0		0	

4.6 Peak Expiratory Flow Rate

We monitored lung function in our patients by measuring peak expiratory flow rate (PEFR). The unit of measurement was litres/minute (l/min). We used either a standard Wright's or mini Wright's peak flow meter. Prior to a measurement we demonstrated the correct technique of use. Measurements were made with the patient standing up and holding the meter horizontally without obstructing movement of the marker, which was set in zero position. Patients were asked to take a deep breath in, hold their breath, put their lips around the mouthpiece ensuring a good seal, and blow as hard and fast as they could into the meter. The reading was recorded; marker reset to zero and after a minutes rest the measurement was repeated. Three such readings were recorded but for the purpose of analysis we took the highest figure.

In the study to characterize patients PEFR was measured pre and post intranasal challenge. If baseline PEFR was < 80% of predicted value the nasal challenge was deferred. Predicted values were calculated from a Nomogram chart for adults (Gregg and Nunn, 1989). A 25% fall in PEFR from baseline was taken as an indicator of a reaction.

In Trial 1, which involved aspirin-sensitive patients, PEFR was monitored closely to detect any deterioration in asthma. In addition to measuring PEFR at every 6-week visit, patients were given a peak flow meter to keep at home, and record daily morning and evening readings. These were entered into a diary, which was used for analysis. In trial 2 (in aspirin tolerant patients), PEFR was measured at every 3-month visit.

4.7 Diary

In Trial 1 we employed a structured diary to collect data from participating patients. They recorded a nasal score, chest score, PEFR, and NIPF on a twice-daily basis. A separate information sheet was provided to aid entering data (Appendix D). In the diary the patients recorded a week's data on each sheet (Appendix D). We provided these diary sheets when the patients attended their 6-weekly visits.

The symptoms taken into consideration while arriving at a nasal score were blockage, rhinorrhoea, itching, sneezing, and sense of smell. A composite score, which was an integer between 0 and 9, was entered twice daily. Similarly, the

symptoms considered for the chest score were tightness of chest, wheeze, shortness of breath on exertion, daytime cough, and night cough. The scoring system was similar to the nasal score (0-9).

Patients were provided with a Youlten's nasal inspiratory peak flow meter and a Mini-Wright's peak flow meter for daily measurements. In the diary they recorded the highest of 3 readings on a twice daily basis.

4.8 Quality of life questionnaire

In Trial 2 we included a quality of life (QOL) questionnaire to study the patients' perceptions of changes in their health status. As our trial population were patients with nasal polyps we made minor modifications to the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ), which is a disease specific questionnaire for patients with allergic rhinoconjunctivitis (Juniper and Guyatt, 1991). Although the changes were not validated, this questionnaire has been used in several trials conducted by our department with permission from Professor Juniper.

The basic structure of the original questionnaire was maintained apart from a domain on eye symptoms, which was excluded (Appendix J). All the original items in each domain were included and new items added after consultation amongst the authors to make it more specific for this study. The questionnaire has 6 main domains with questions within them, which inquire about the effects of nasal polyps on the patients' physical and psychosocial well-being. The 6 main domains with number of items within each () were: nasal problems (4), other symptoms (9), sleep disturbances (5), practical problems (5), interference with three patient-chosen activities (3), and emotional state (6). Each question is answered on a scale of 0 to 6; a higher score indicating poorer functioning or deteriorating symptoms.

Each patient completed the questionnaire (interviewer supervised) at the start of the trial and at every subsequent visit. For all domains they reported the degree of difficulty experienced over a 1-week period preceding their visit.

4.9 Olfactory function

In Trial 2 we used the University of Pennsylvania Smell Identification Test (UPSIT) to measure olfaction. It has 40 microencapsulated odours, each on a separate

'scratch-and-sniff' pad (Doty et al. 1984). Patients' were provided with a sharp object to scratch the pad and respond by choosing from 4 given alternatives. Between each 'scratch' the patient was instructed to clean the object so as to avoid contamination of the next pad. The correct method of scoring involves patients' making a guess even if they are unable to identify an item. This test has a good short-term and long-term test-retest reliability.

4.10 Nasal Biopsy

A nasal biopsy was taken from the inferior turbinate of aspirin-sensitive patients in Trial 1. The biopsy was performed at the start and end of the first phase, and at the end of the second phase (Figure 3.1). Patients were requested and had a choice to undergo the procedure. Following clinical examination a suitable site was chosen to biopsy. Cotton wool soaked in 10% cocaine solution with 1:1000 epinephrine was placed on the site and left for 20 minutes. Biopsies were taken with Gerritsma forceps (Fokkens et al. 1988). These forceps provide an adequate biopsy measuring 2.5 mm^2 in diameter with undamaged epithelium and sufficient depth of lamina propria. The procedure is quick, easy to perform, and causes minimal discomfort. This latter feature encourages acceptance of the procedure, particularly when repeated biopsies have to be performed. After taking a biopsy the site was covered with dry cotton wool for tamponade. After 30 minutes of observation for any further bleeding patients were discharged.

Nasal biopsies were also performed on aspirin tolerant individuals with nasal polyps and processed in an identical manner to those from aspirin-sensitive patients, thus allowing comparisons. Aspirin tolerant patients, from the database we created following lysine-aspirin nasal challenges, were invited to have a biopsy. It was not essential for these patients to be in Trial 2. Patients from Trial 2 who agreed to have a biopsy had the procedure after completing the trial.

The biopsy specimen was immediately transferred into the centre of a small tin foil receptacle. A preservative was poured on the specimen to cover it completely and fill the receptacle. The biopsy was snap frozen by immersing it in liquid nitrogen. The frozen receptacle was transferred into a small plastic container, which was labeled to allow identification of the patient and the date of biopsy. It was stored in liquid nitrogen until immunohistochemical analysis.

4.11 Methods for Laboratory studies

4.11.1 *Immunohistochemistry of nasal biopsies*

Five μm sections were cut from the biopsy specimens, and mounted on to slides coated with 3-amino propyl triethoxysilane (Sigma, Dorset, UK), fixed for 10 minutes in acetone, and stored at -70^0 C until used for immunohistochemistry.

4.11.1a *Detection of cells expressing CysLT$_1$ and B-LT receptors*

Polyclonal rabbit antibodies against the CysLT$_1$ or the B-LT receptors (Cayman Chemicals, MI, USA) were used. The manufacturer has extensively validated these antibodies. For example, in COS-7 cells transfected with myc-tagged CysLT$_1$ receptor, the polyclonal antibody showed co-localisation of staining with anti-myc using confocal microscopy. Non-transfected cells, B-LT receptor transfected cells or cells transfected with vector control showed no staining. Conversely, using confocal microscopy the polyclonal B-LT receptor antibody was shown to stain COS-7 cells transfected with the B-LT receptor, but not cells transfected with CysLT$_1$ receptor or vector control. The immunostaining avidin biotin protocol was used as described previously (Nasser et al. 1996; Sousa et al. 1997a). Briefly, primary antibodies were used at a dilution of 1/50 and 1/600 for CysLT$_1$ and B-LT, respectively. Polyclonals were developed with a swine anti-rabbit biotinylated secondary antibody (Dako, Ely, UK) and the immunoperoxidase brown colour reaction developed with diaminobenzidine (DAB) (Sigma). Endogenous biotin reactivity and peroxidase activity were abolished as previously described (Nasser et al. 1996; Sousa et al. 1997a).

4.11.1b *Detection of total cells expressing CD45*

Sections were stained with murine monoclonal anti-human CD45 primary antibody, then peroxidase-conjugated second layer rabbit anti-mouse antibody (Dako) used at 1/400 dilution, and a brown colour reaction developed with DAB.

4.11.1c *Phenotype of cells expressing $CysLT_1$ and B-LT receptors*

Sequential immunostaining for cells expressing $CysLT_1$ and B-LT receptors and leukocyte phenotypic markers was performed as previously described (Nasser et al. 1996; Sousa et al. 1997a). Briefly, the murine monoclonal antibodies UCHT1 (anti-CD3, Dako, used at 1/50 dilution), PGM1 (anti-CD68, Dako, 1/100 dilution), EG2 (anti-eosinophil cationic protein, Pharmacia, 1/100 dilution), AAI (anti-mast cell tryptase, Dako, 1/50 dilution), NP57 (anti-neutrophil elastase, Dako, 1/100 dilution) were used to identify T cells, macrophages, activated eosinophils, mast cells and neutrophils respectively. Alkaline phosphatase conjugated goat anti-mouse secondary antibody (Seralab, Salisbury, UK used at 1/100 dilution) was used to detect these antibodies. A blue colour reaction was developed with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Sigma). The rabbit polyclonal antibodies were detected as described above. Double-stained cells were identified which expressed both a blue and a brown precipitate.

4.11.1d *Cell counting*

Sections were coded and counted by an investigator without prior knowledge of the randomisation protocol. Positive cells were counted using an Olympus microscope connected to an image analyser and associated software (Zeiss KSS 300, Imaging Associates, Oxford, UK). Positive staining cells were counted in the total area of the biopsies, which was measured automatically by the analyser. Cell counts were expressed per square mm of tissue section.

The following study was a collaborative project with Professor Jane Mitchell's group (Department of Critical Care Medicine, Imperial College School of Medicine, National Heart and Lung Institute), and she has kindly provided details of the methods used for analysis of the specimens.

4.11.3 Measurement of nitric oxide synthase (NOS) activity in nasal polyps

Samples were collected and stored in liquid nitrogen until NOS activity was measured by the ability of tissue homogenates to convert tritiated L-arginine to L-citrulline in a L-N^G nitro-L-arginine (L-NAME) inhibitable fashion. Samples were given numbers and laboratory scientists were blinded to the patients clinical characteristics or grouping during analysis for iNOS activity. Briefly, tissue was homogenized on ice in 50 mM Tris buffer (pH 7.4) containing phenylmethylsufonyl fluoride (1mM) in a ratio of 5:1 (vol:wt). The NOS activity was measured in the presence of the co-factors tetrahydrobiopterin (5μM), NADPH (1mM), calmodulin (300U/ml). L-valine (10mM) was added to inhibit the conversion of L-arginine to L-citrulline via the arginase pathway. NOS activity was measured in each sample in the presence of calcium (2mM) and also in the absence of calcium but in the presence of EGTA (1mM). Separate incubations were carried out with each sample in the presence of the NOS inhibitor L-NAME (1mM), activity remaining in these incubations were considered to be not specific to the NOS pathway. In each case the reaction was initiated by the addition of substrate, L-arginine (10μM plus 0.03μM tritiated). After 30 min incubation at room temperature the reaction was stopped by the addition of ice-cold 20mM HEPES buffer (pH 5.5). The newly formed citrulline was separated from the arginine by passing the reaction solution of Dowex-50W (sodium form) columns and eluted with the HEPES buffer, scintillation fluid was then added and samples counted in a scintillation counter.

4.12 Statistical methods

Collected data was entered into a statistical package – SPSS for Windows version 10.0.7. This statistical package was used for analysis and production of some graphs. Graphs for the trials and biopsy results were produced using Graph Pad Prism version 4. Details of sample size calculations and analysis of outcome measures has

been included in the relevant sections. I consulted Professor S. Senn from University College London for analysis of the two clinical trials.

Non-parametric tests have been used for analysis of data. Thus, all graphs will show medians. Within group analysis was done using Wilcoxon signed ranks test, and between group analysis was done using Mann Whitney U test. Significance level for all results was set at $p < .05$.

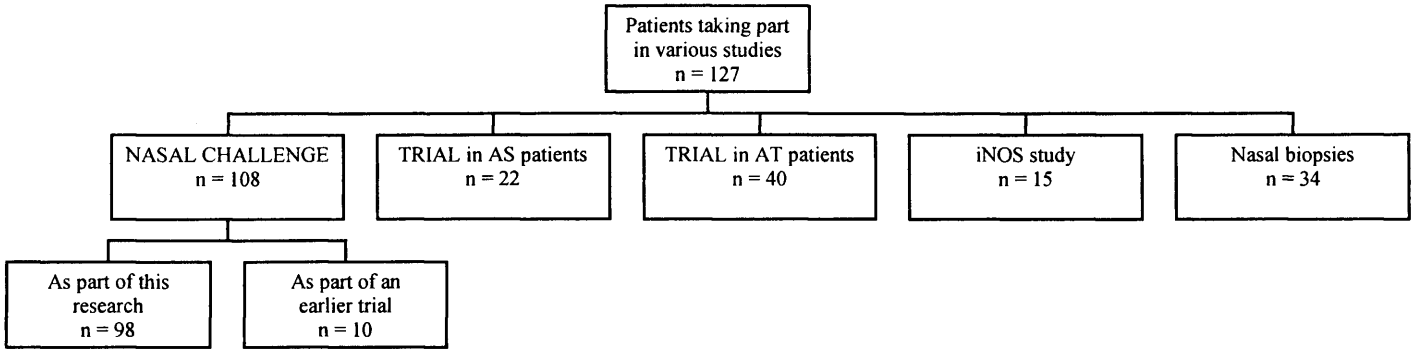
4.13 Ethical approval

Trial protocols were presented to the local ethics committee at the RNTNEH. These outlined all interventions that patients would undergo. They were nasal challenge with lysine-aspirin, exposure to placebo or LAS during the trials, nasal biopsy, and nasal polypectomy. Patient information sheet, and consent forms were also attached to the protocols. Formal approval for the trials, and all interventions was received from the ethics committee.

RESULTS

One hundred and twenty-seven patients with nasal polyps were involved in the various studies (Figure 5.1, Table 5.1). These included 70 males (55.1%) and 57 females (44.9%) with an age range of 20-73 years (mean ± S.D = 44.8 ± 11.6). Skin prick tests were done in 117 patients and results were positive in 77 patients (77/117, 65.8%). A history of asthma requiring regular medication was obtained from 77 patients (77/127, 60.6%). Thirty-nine patients (39/127, 30.7%) gave a history of an aspirin induced reaction. The majority of these patients had undergone surgery for polyps (97/127, 76.4%), with the number of operations ranging from 1-20 (mean ± S.D = 3.6 ± 3.4).

Figure 5.1; Table 5.1 **OVERVIEW OF TRIALS AND STUDIES**



	All studies	Nasal challenge	Trial 1 in AS patients	Trial 2 in AT patients	iNOS study	Biopsy study
Age in years: range (mean±S.D)	20-73 (44.8±11.6)	20-69 (43.8±11.2)	23-68 (41.2±11.9)	28-73 (47.1±10.6)	35-60 (43.3±8.5)	20-69 (43.9±14.3)
Sex: Male (Female)	70 (57)	55 (43)	7 (15)	28 (12)	6 (9)	14 (20)
Asthma: Yes (No)	77 (50)	64 (33)	18 (4)	21 (19)	8 (7)	25 (9)
History of AS: Yes (No)	39 (87)	36 (45)	19 (3)	0 (40)	5 (10)	22 (12)
Challenge: +ve (-ve)	29 (79)	27 (71)	20 (2)	1 (34)	5 (0)	22 (6)
Skin test: +ve (-ve)	77 (40)	63 (29)	16 (5)	23 (16)	10 (2)	20 (10)
Surgery: Yes (No)	97 (30)	74 (24)	19 (3)	34 (6)	15	24 (10)
Operations for polyps: range (mean±S.D)	1-20 (3.6±3.4)	1-20 (2.7±3.4)	1-20 (5.2±4.5)	1-16 (3.3±3.6)	1-7 (2.4±2.1)	1-20 (5.4±4.2)

5.1 Nasal challenge results

5.1.1 *Patients*

One hundred and eight patients with nasal polyps (108/127, 85%) underwent an intranasal lysine-aspirin challenge. Of these, 10/108 (9.3%) were challenged in 1993-95 as part of a previous study. The results of this study have been published (Scadding et al. 1995). Thus, the results of the remaining 98/108 (90.7%) intranasal lysine-aspirin challenges are presented.

Challenges were performed in 98 patients (M = 55, F = 43, ratio = 1.28:1), with an age range of 20-69 years (mean ± S.D = 43.8 ± 11.2). The majority of these patients had asthma (64/98; 65.3%), were skin prick test positive (63/98; 63.4%), and had undergone surgery for their nasal polyps (74/98; 75.5%).

All patients with asthma were on a combination of inhaled corticosteroids (preventer), and a bronchodilator (reliever). Their lung function was well controlled on this combination and none of our patients were on regular oral corticosteroids. The commonest combination used amongst our patient population was beclomethasone dipropionate plus salbutamol.

In patients who had undergone surgery the number of interventions ranged from 1-20 (mean ± S.D = 2.7 ± 3.4). Intranasal polypectomy was the commonest operation performed in 63/98 (64.3%) patients. Endoscopic polypectomy was performed in 16/98 (16.3%) patients. Four patients (4/98, 4.1%) had undergone radical surgery for control of their polyp disease. Two of these had a Caldwell-Luc operation and 2 had an external ethmoidectomy procedure.

All patients were asked about the effect of aspirin or NSAID intake. Those patients who recalled tolerating aspirin or NSAID were specifically asked if they had taken either drug during the past year. If they could not recall taking the drug during the past year their responses were considered equivocal. Thirty-six patients (36/98, 36.7%) gave a history of an adverse reaction; forty-five patients (45/98, 45.9%) gave a history of tolerating aspirin or NSAID intake, and 17 patients (17.3%) could not recall having ingested aspirin or NSAID for a substantial period of time. These patients were considered in the equivocal group.

Of the 36 patients with a history of aspirin induced adverse reaction 30 (83%) developed asthma (19 only asthma; 9 with urticaria and angioedema, 2 with

rhinoconjunctivitis). Two patients developed nasal symptoms only, and 4 only urticaria.

5.1.2 *Changes in measured parameters following challenge*

5.1.2a Acoustic rhinometry measurements

i) Volume

We had complete data on 96/98 (97.9%) patients. The average pre and post challenge values were 16.8 ± 9.3 mls (median ± S.D) (range = 3.5 to 56.2 mls), and 15.8 ± 9.4 mls (range = 2.2 to 51.9 mls) respectively.

The change following challenge was calculated by subtracting the pre from the post value. Thus, a negative (-) change indicated deterioration. The average change in volume was -.94 ± 4.8 mls (median ± S.D) (range = -20.7 to 17.0 mls). The average *percentage* change was -6.9 ± 22.6 mls (range = -70.7 to 61.1 mls).

ii) Minimum cross-sectional area 1 ($Amin_1$)

We had complete data on 97/98 (98.9%) patients. The average pre and post challenge values were 1.15 ± .56 mm^2 (median ± S.D) (range = .20 to 3.01 mm^2), and 1.08 ± .59 mm^2 (range = .06 to 3.62 mm^2) respectively.

The change following challenge was calculated by subtracting the pre value from the post value. Thus, a negative (-) change indicated deterioration. The average change in $Amin_1$ was -.1 ± 0.26 mm^2 (median ± S.D) (range = -1.07 to 0.61 mm^2). The average *percentage* change was -8.2 ± 22.7 mm^2 (range = -72.67 to 46.72 mm^2).

iii) Minimum cross-sectional area 2 ($Amin_2$)

We had complete data on 78/98 (78.9%) patients. The average pre and post challenge values were 1.98 ± 2.28 mm^2 (median ± S.D) (range = .39 to 11.93 mm^2), and 1.76 ± 2.59 mm^2 (range = .25 to 14.37 mm^2) respectively.

The change following challenge was calculated by subtracting the pre value from the post value. Thus, a negative sign (-) indicated deterioration. The average change in

$Amin_2$ was -.09 ± 1.01 mm^2 (median ± S.D) (range = -3.21 to 4.69 mm^2). The average *percentage* change was –5.21 ± 11.65 mm^2 (range = -75.76 to 76.52 mm^2).

5.1.2b Nasal inspiratory flow rate

We had complete data on 78/98 (79.6%) patients. The average pre and post challenge values were 157.5 ± 70.1 lts/min (median ± S.D) (range = 0 to 340 lts/min), and 140 ± 72.3 lts/min (range = 0 to 300 lts/min) respectively.

The change following challenge was calculated by subtracting the pre value from the post value. Thus, a negative sign (-) indicated deterioration. The average change in nasal inspiratory flow rate was -.0 ± 37.9 lts/min (median ± S.D) (range = -160.0 to 50.0 lts/min). The average *percentage* change was -2.1 ± 27.6 lts/min (range = -100.0 to 57.14 lts/min).

5.1.2c Visual analogue scale

Sneezing, itching, obstruction, and rhinorrhoea were the 4 symptoms marked on a scale of 0 – 10. Thus, the lowest and highest possible scores were 0 and 40 respectively. Scales were marked prior to and post challenge providing us with paired data for comparison.

We had 93/98 (94.9%) complete pairs of data. The average pre and post challenge scores were 5 ± 5.58 (median ± S.D; range = 0 to 26.9), and 6 ± 5.81 (range = 0 to 24). Change was calculated by subtracting post from pre values. Thus a negative (-) sign indicated deterioration. The average change was 0 ± 4.17 (range = -16.5 to 10.5).

5.1.2d Peak expiratory flow rate

We had complete data on 95/98 (96.9%) patients. The average pre and post challenge values were 470 ± 110.5 lts/min (median ± S.D) (range = 210 to 700 lts/min), and 470 ± 108.9 lts/min (range = 210 to 680 lts/min) respectively. The change following challenge was calculated by subtracting the pre value from the post value. Thus, a negative sign (-) indicated deterioration. The average change in peak expiratory flow rate was -5 ± 27.1 lts/min (median ± S.D; range = -80.0 to 80.0 lts/min). The average

percentage change for this group was -.88 ± 7.1 lts/min (range = -24.6 to 34.8 lts/min).

5.1.3 *Statistical analysis to classify a challenge as positive - Receiver Operating Characteristic (ROC) curve*

The utility of each measured parameter towards diagnosing aspirin-sensitivity was examined by plotting ROC curves.

i. Explanation of ROC curves

ROC curves are a way to analyse the accuracy of a diagnostic test (Rao, 2003), (Zweig and Campbell, 1993). The accuracy of a diagnostic test depends on its ability to detect disease when truly present (sensitivity), and to recognise disease absence when it is truly absent (specificity). A ROC curve is a graphical representation displaying the sensitivity of a diagnostic test over all possible false positive rates (1 – specificity). It shows that any increase in sensitivity is accompanied by a decrease in specificity.

Once a curve is plotted it also provides us with various 'cut-off' points each having their own sensitivity and specificity. With the curve we can choose the best cut-off value for distinguishing between positive and negative results or find the sensitivity/specificity for any pre-determined cut-off value. Another useful indicator of the discriminative value of the diagnostic test is area under the ROC curve (AUC_{ROC}). An area of 1 represents a perfect test, whereas an area of .5 represents a worthless test. A rough guide for classifying the accuracy of a diagnostic test is shown in Table 5.2.

ii. Constructing a ROC curve

In the absence of a gold standard criterion for diagnosis of a disease, investigators have used patient reported symptoms or signs as a substitute in ROC analysis (Christian Wasmuth et al, 2002). Thus, we used a positive history of aspirin-induced reaction to plot the ROC curve for each measured parameter. Statistical software Graphpad Prism (v 4.00) was used to construct the plot, give an AUC_{ROC}, and provide sensitivity/specificity values for the cut-off point chosen (Table 5.3). Results above this cut-off point would signify a positive challenge.

5.1.3a *ROC curve of Volume measurements* (Figure 5.2)

For this acoustic rhinometry measurement we chose the cut-off point as -25%. The coefficient of variation for volume measurements in our laboratory was approximately 10%. Hence, we considered a reading of -25% or above as a valid cut-off to denote a positive challenge. Also, this threshold has been evaluated in an earlier study, and it provides good sensitivity/specificity (Casadevall et al, 2000).

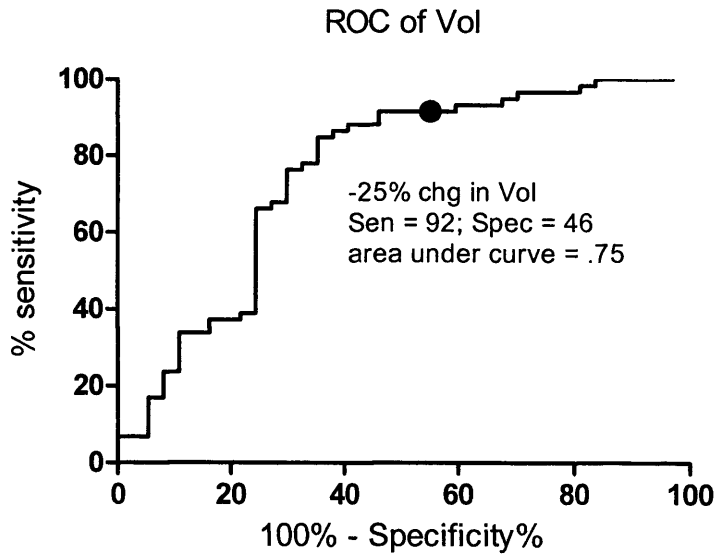
Overall accuracy of the test was fair as shown by area under the curve (AUC_{ROC} = .75; 95% CI = .65 - .86). A fall in nasal volume of 25% or more gave us a sensitivity of 92% (95% CI = 81 – 97), and a specificity of 46% (95% CI = 29 – 63).

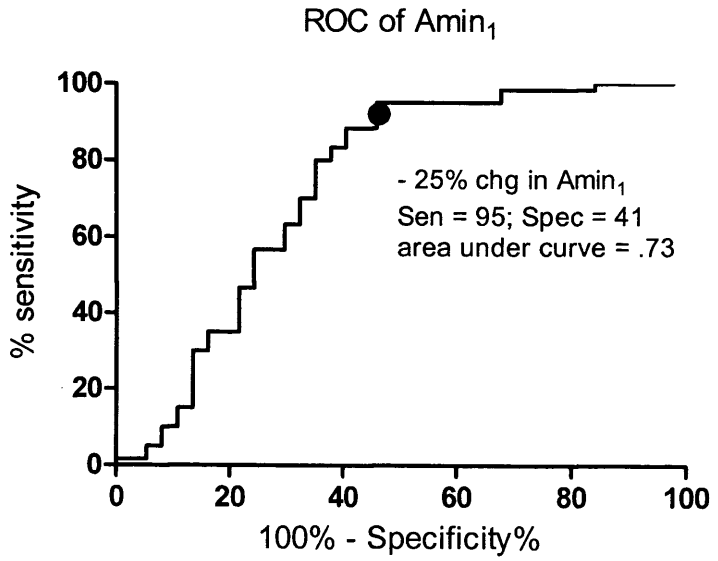
5.1.3b *ROC curve of $Amin_1$ measurements* (Figure 5.2)

For $Amin_1$ we chose the cut-off point as -25%. In our laboratory the coefficient of variation for $Amin_1$ was approximately 10%. Hence, we considered a reading of -25% or above as a valid cut-off to denote a positive challenge.

Overall accuracy of the test was fair as shown by area under the curve (AUC_{ROC} = .73; 95% CI = .62 - .85). A fall in $Amin_1$ of 25% or more gave us a sensitivity of 95% (95% CI = 86 – 99), and a specificity of 41% (95% CI = 25 – 58).

Figure 5.2


ROC of Vol
100
80
60
40
20
0
% sensitivity
-25% chg in Vol
Sen = 92; Spec = 46
area under curve = .75
0
20
40
60
80
100
100% - Specificity%


ROC of Amin1
100
80
60
40
20
0
% sensitivity
- 25% chg in Amin1
Sen = 95; Spec = 41
area under curve = .73
0
20
40
60
80
100
100% - Specificity%

5.1.3c *ROC curve of $Amin_2$ measurements* (Figure 5.3)

For $Amin_2$ we chose the same cut-off point as $Amin_1$ (-25%).

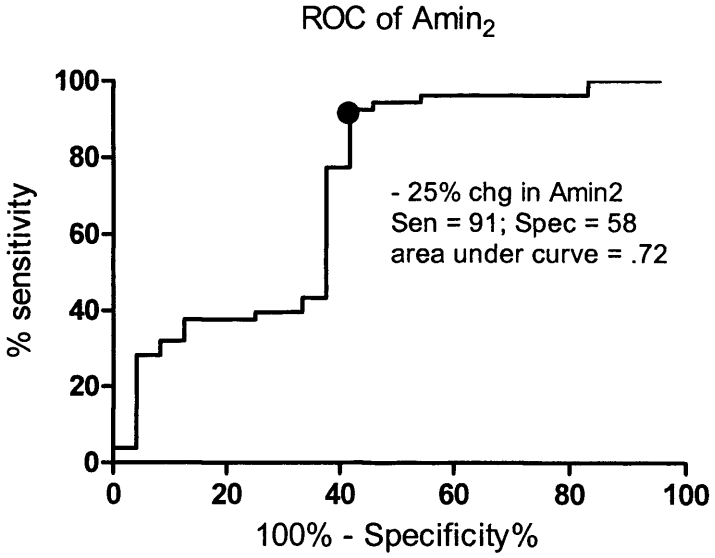
Overall accuracy of the test was fair as shown by area under the curve (AUC_{ROC} = .72; 95% CI = .59 - .86). A fall in $Amin_2$ of 25% or more gave us a sensitivity of 91% (95% CI = 79 – 97), and a specificity of 58% (95% CI = 37 – 78).

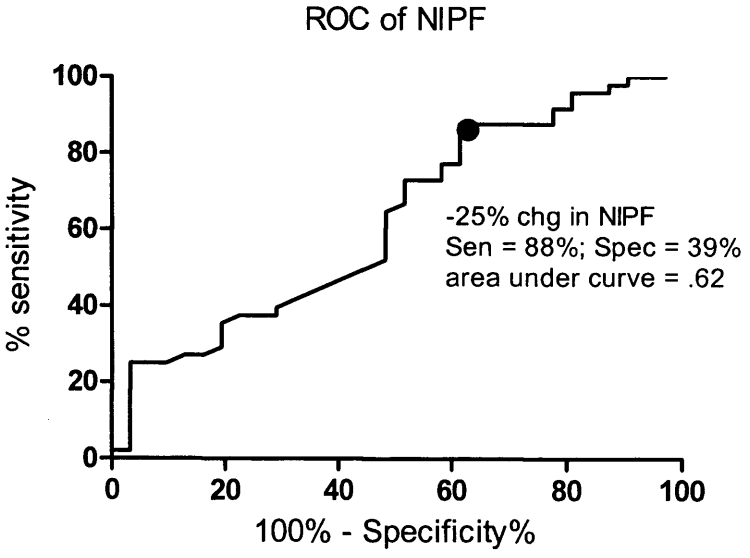
5.1.3d *ROC curve of NIPF measurements* (Figure 5.3)

For NIPF we chose the cut-off point as -25%. The coefficient of variation for this measurement has been shown to vary from 6% to 10% (Holmstrom et al, 1990), (Lee D et al, 2004).

Overall accuracy of the test was poor as shown by area under the curve (AUC_{ROC} = .62; 95% CI = .45 - .79). A fall in NIPF of 25% or more gave us a sensitivity of 88% (95% CI = 75 – 95), and a specificity of 39% (95% CI = 22 – 58).

Figure 5.3


ROC of Amin2
100
80
60
40
20
0
% sensitivity
- 25% chg in Amin2
Sen = 91; Spec = 58
area under curve = .72
0
20
40
60
80
100
100% - Specificity%


ROC of NIPF
100
80
60
40
20
0
% sensitivity
-25% chg in NIPF
Sen = 88%; Spec = 39%
area under curve = .62
0
20
40
60
80
100
100% - Specificity%

5.1.3e *ROC curve of Visual analogue scale - VAS* (Figure 5.4)

An increase in the composite score by a minimum of 10 points was taken as the cut-off point. VAS is a subjective measurement, but with a minimum score of 0 and maximum possible of 40, a change of 10 was thought as significant.

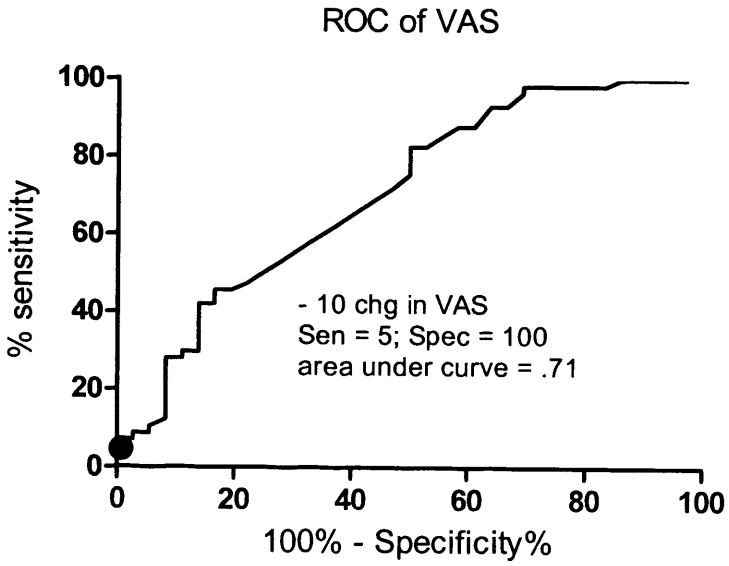
Overall accuracy of the test was fair as shown by area under the curve (AUC_{ROC} = .71; 95% CI = .59 - .82). A 10-point increase in VAS gave us a poor sensitivity of 5% (95% CI = 1.1 – 14.6), but a perfect specificity of 100% (95% CI = 90 – 100).

5.1.3f *ROC curve of PEFR* (Figure 5.4)

A decrease in FEV_1 of 20% is considered significant as a response to inhalation challenge (Nasser et al, 1996), (Nizankowska et al, 2000). We chose -25% as a cut-off to increase the accuracy of our results.

Overall accuracy of the test was poor as shown by area under the curve (AUC_{ROC} = .5; 95% CI = .38 - .62).

Figure 5.4


ROC of VAS
% sensitivity
100
80
60
40
20
0
0
20
40
60
80
100
100% - Specificity%
- 10 chg in VAS
Sen = 5; Spec = 100
area under curve = .71

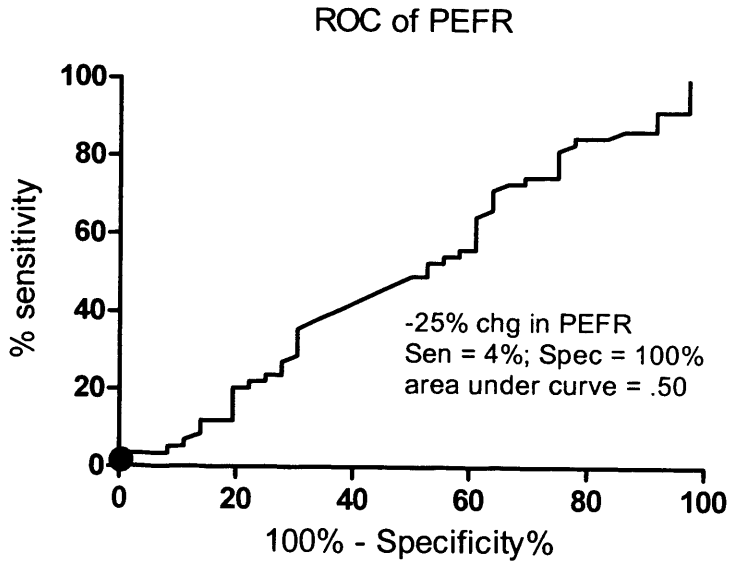

ROC of PEFR
% sensitivity
100
80
60
40
20
0
0
20
40
60
80
100
100% - Specificity%
-25% chg in PEFR
Sen = 4%; Spec = 100%
area under curve = .50

Table 5.2

Accuracy of ROC curves

Area under the ROC curve	Accuracy of test
.9 – 1	Excellent
.8 - .9	Good
.7 - .8	Fair
.6 - .7	Poor
.5 - .6	Fail

Table 5.3

Sensitivity and Specificity derived from ROC curves

Parameter	Cut-off point	Sensitivity (%)	Specificity (%)	Area under ROC
Volume	-25%	92	46	.75
$Amin_1$	-25%	95	41	.73
$Amin_2$	-25%	91	58	.72
NIPF	-25%	88	40	.62
VAS	-10 point change	5	100	.70
PEFR	-25%	4	100	.50

5.1.4 *Challenge results*

Twenty-seven patients (27/98, 27.6%) fulfilled our criteria for a positive challenge, and hence were considered aspirin-sensitive. Figures 5.5, 5.6 show the results of these 27 patients. Measurements shown are Volume, $Amin_1$, $Amin_2$, and NIPF (see legend). PEFR did not deteriorate in any patient by -25%, and thus has been omitted from the graphical presentation of challenge results. Only 7 patients showed a 10-point deterioration in their composite VAS score. These patients have been highlighted in the graphs by an asterisk (*).

Seventy-one patients did not fulfill our criteria for a positive challenge, and hence were labeled aspirin tolerant. Figures 5.7, 5.8, 5.9, 5.10 show their results. Measurements shown are Volume, $Amin_1$, $Amin_2$, and NIPF (see legend). PEFR did not deteriorate in any patient by -25%, and thus has been omitted from the graphical presentation of challenge results. None of the patients had a significant change in VAS score.

Figure 5.5

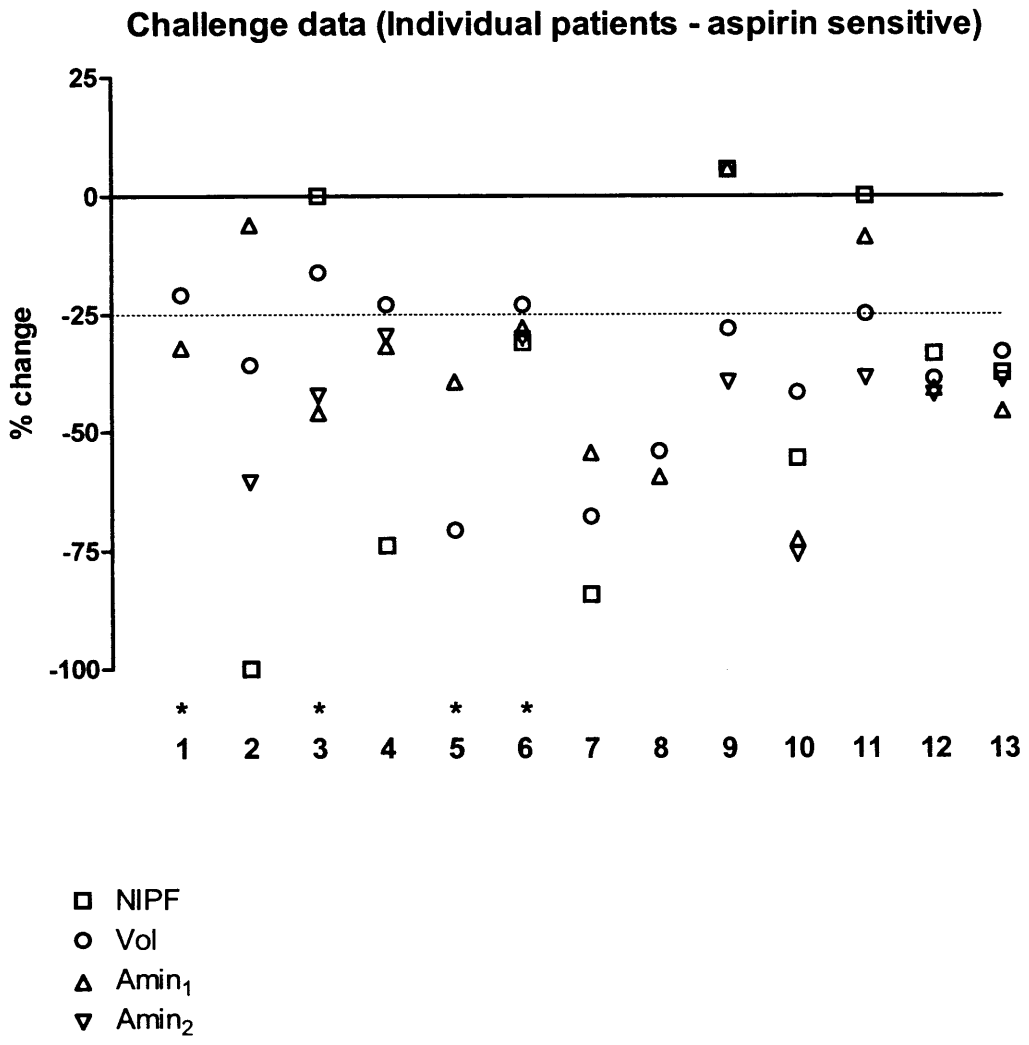

Challenge data (Individual patients - aspirin sensitive)
25
0
-25
-50
-75
-100
% change
1
2
3
4
5
6
7
8
9
10
11
12
13
NIPF
Vol
Amin1
Amin2

Figure 5.6

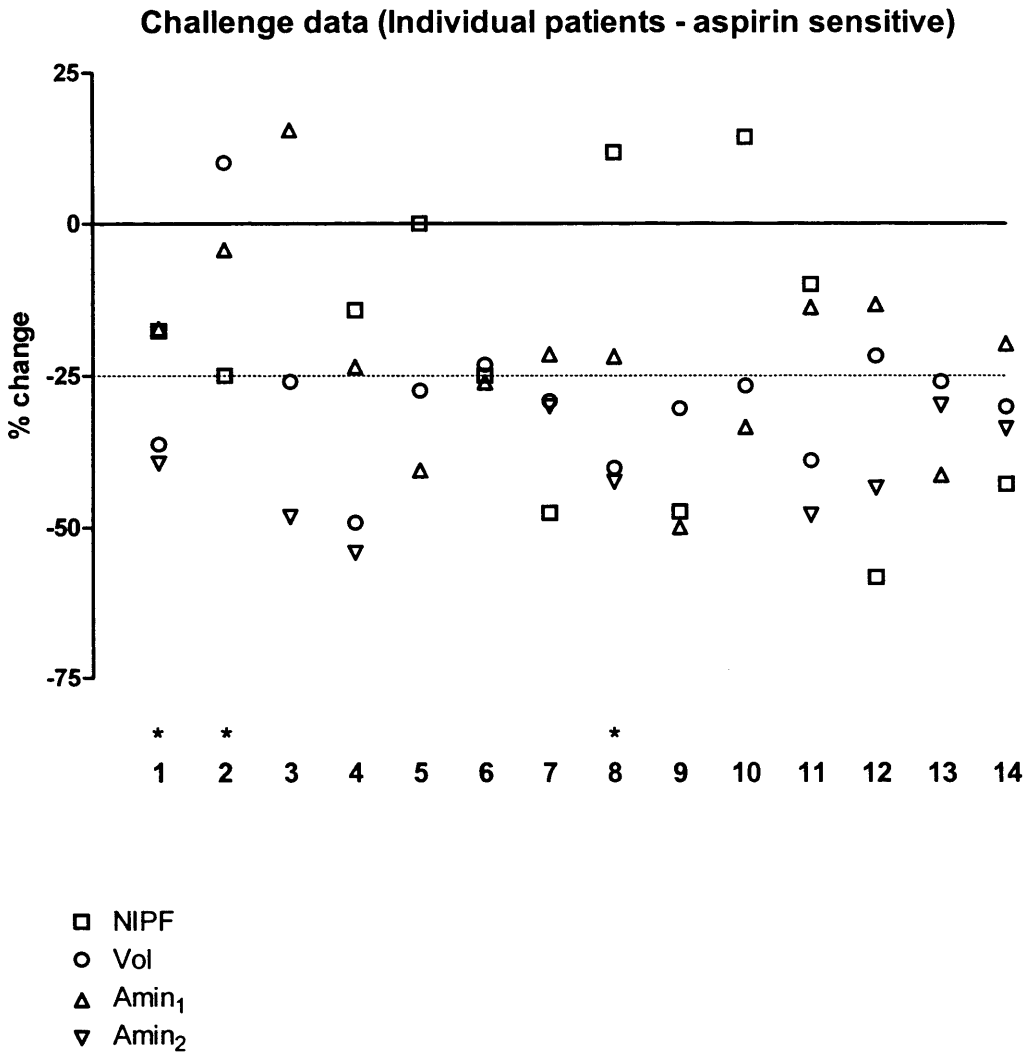

Challenge data (Individual patients - aspirin sensitive)
% change
25
0
-25
-50
-75
1
2
3
4
5
6
7
8
9
10
11
12
13
14
NIPF
Vol
Amin1
Amin2

Figure 5.7

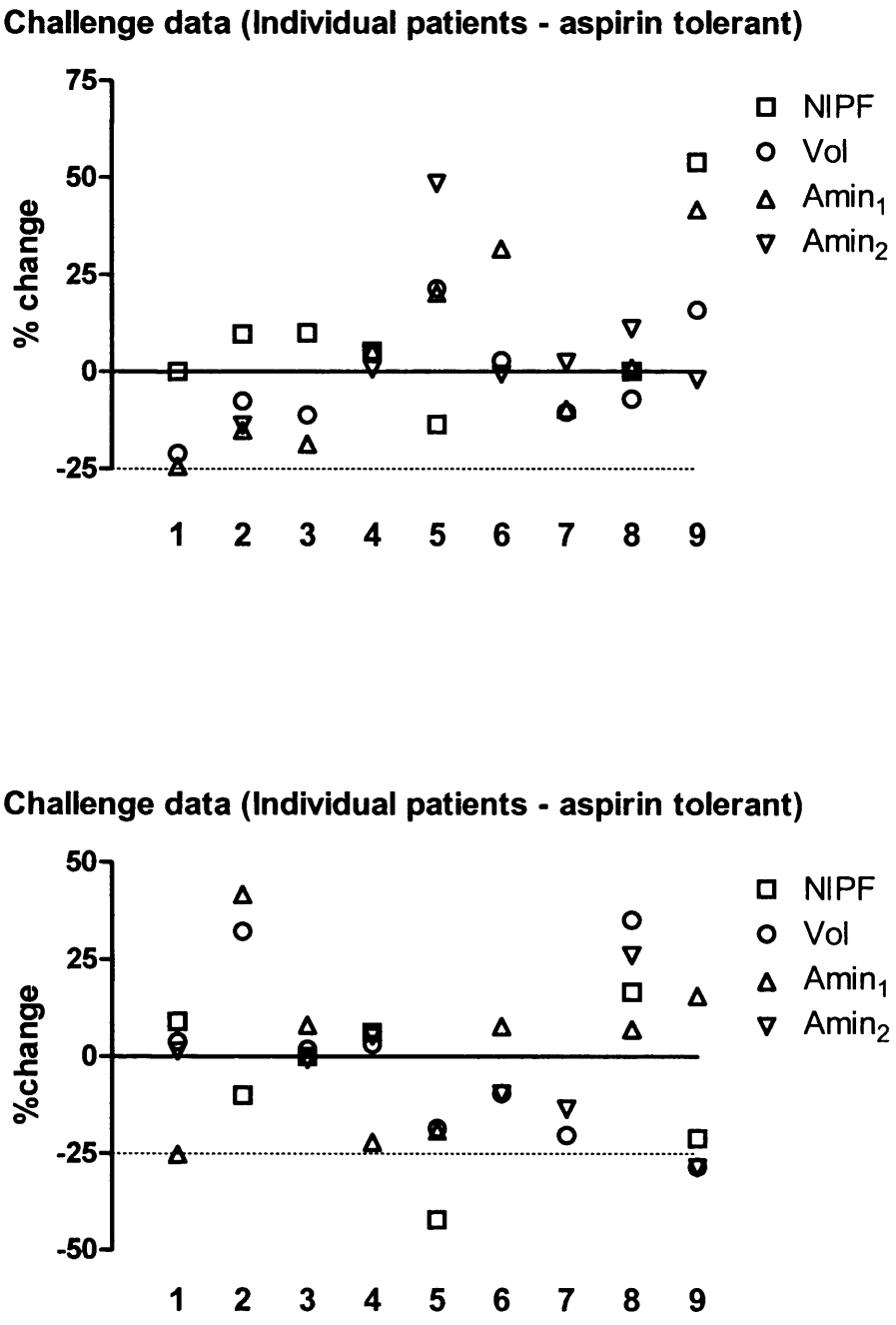
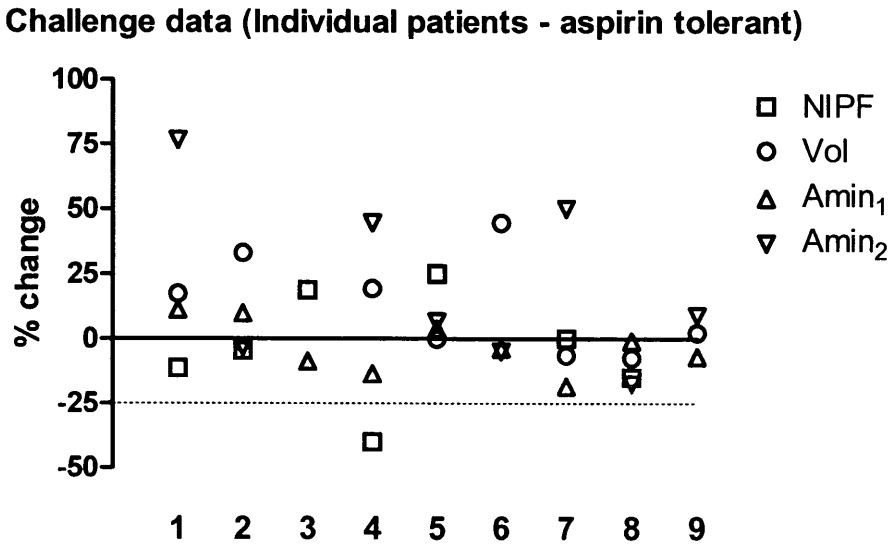

Challenge data (Individual patients - aspirin tolerant)
% change
75
50
25
0
-25
1 2 3 4 5 6 7 8 9
NIPF
Vol
Amin1
Amin2
Challenge data (Individual patients - aspirin tolerant)
%change
50
25
0
-25
-50
1 2 3 4 5 6 7 8 9
NIPF
Vol
Amin1
Amin2

Figure 5.8


Challenge data (Individual patients - aspirin tolerant)
100
75
50
25
0
-25
-50
% change
1 2 3 4 5 6 7 8 9
NIPF
Vol
Amin1
Amin2

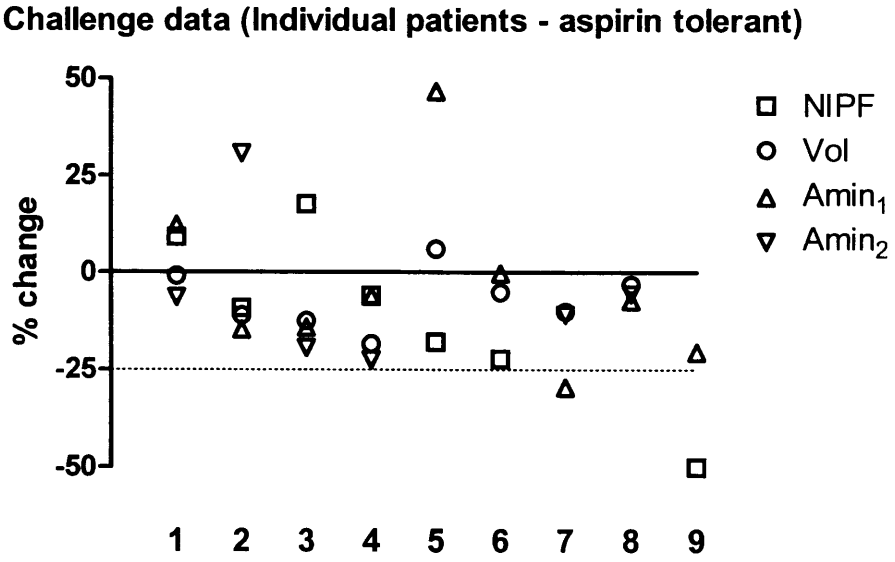
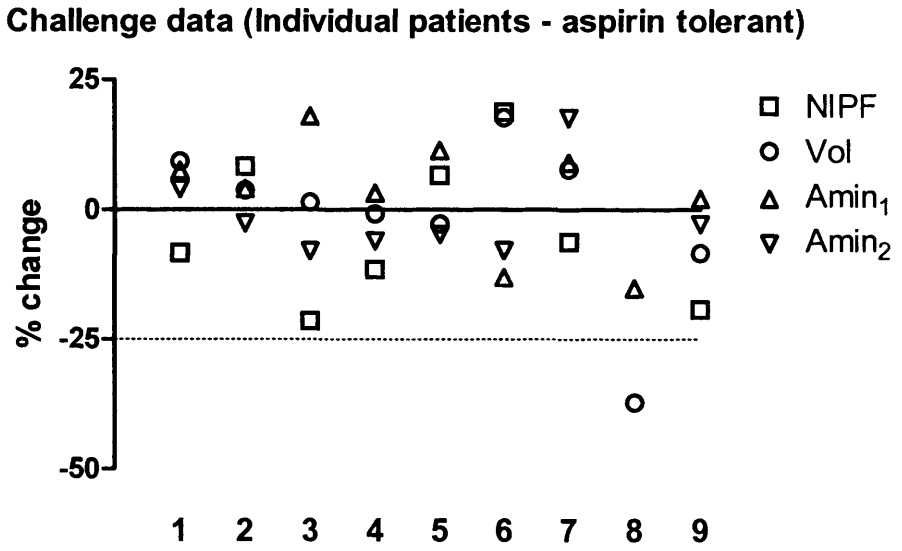

Challenge data (Individual patients - aspirin tolerant)
50
25
0
-25
-50
% change
1 2 3 4 5 6 7 8 9
NIPF
Vol
Amin1
Amin2

Figure 5.9


Challenge data (Individual patients - aspirin tolerant)
% change
25
0
-25
-50
1 2 3 4 5 6 7 8 9
NIPF
Vol
Amin1
Amin2

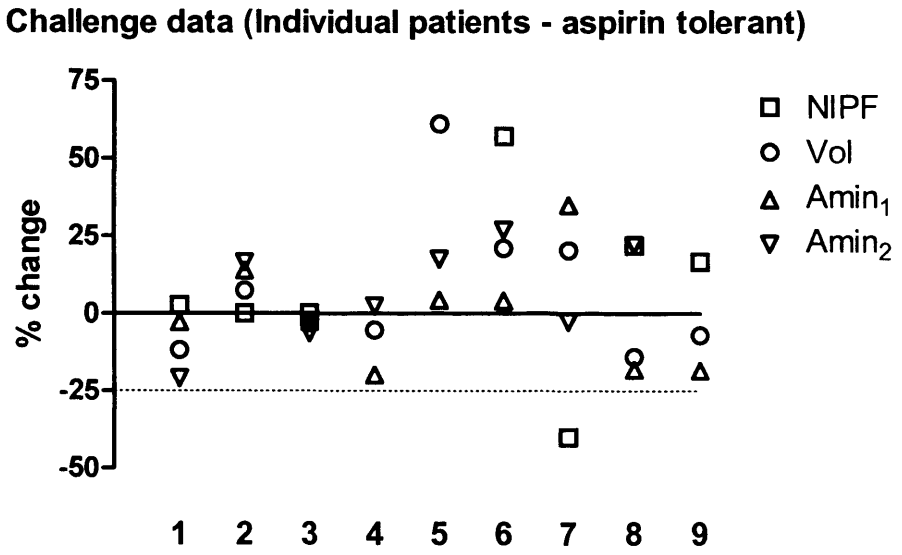
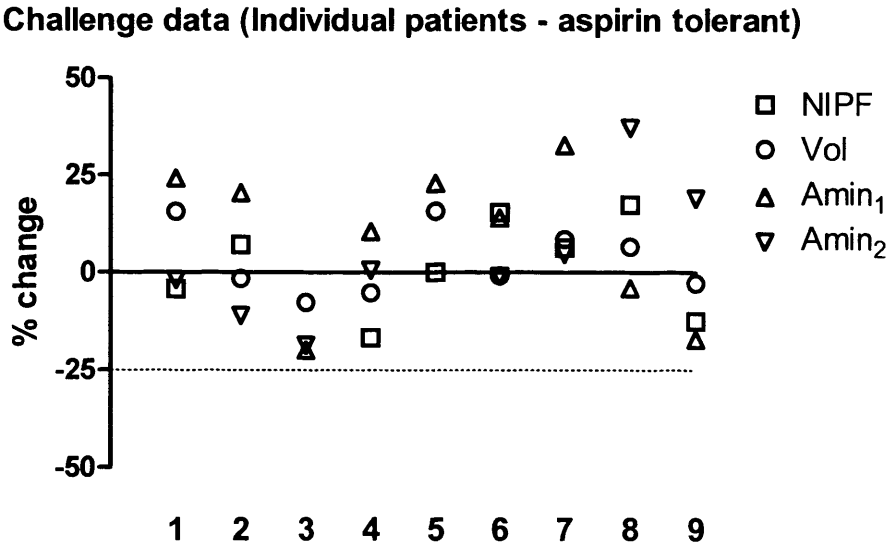
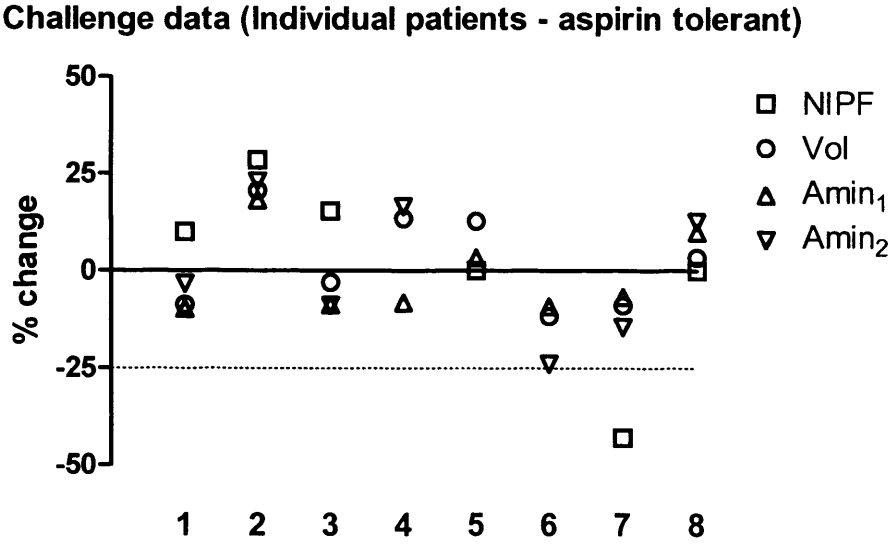

Challenge data (Individual patients - aspirin tolerant)
% change
75
50
25
0
-25
-50
1 2 3 4 5 6 7 8 9
NIPF
Vol
Amin1
Amin2

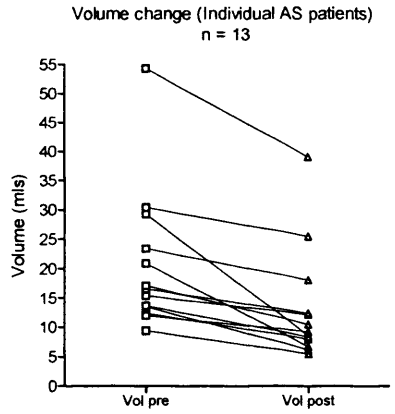
Figure 5.10

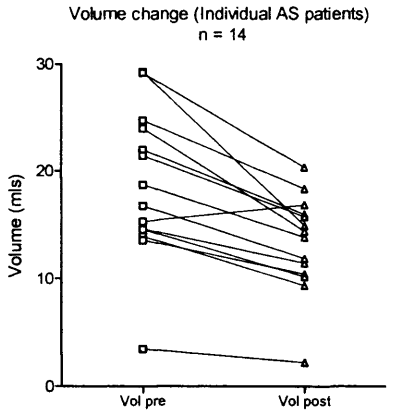

Challenge data (Individual patients - aspirin tolerant)
% change
50
25
0
-25
-50
1 2 3 4 5 6 7 8 9
NIPF
Vol
Amin1
Amin2

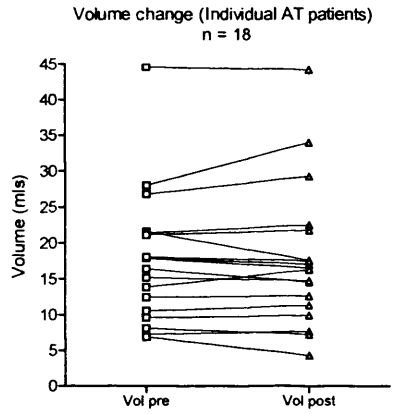

Challenge data (Individual patients - aspirin tolerant)
% change
50
25
0
-25
-50
1 2 3 4 5 6 7 8
NIPF
Vol
Amin1
Amin2

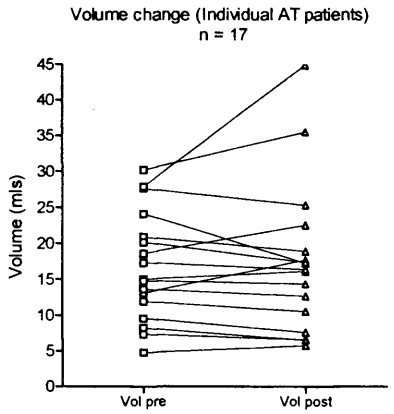
Figures on the previous pages show percentage changes for each parameter and in each individual. Further representations of the changes in the various parameters for each individual patient are shown in the before-after graphs (Figures 5.11, 5.12, 5.13, 5.14, 5.15, and 5.16).

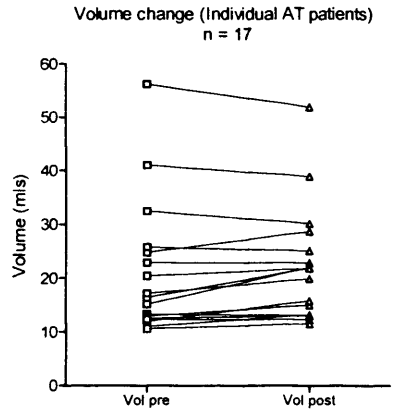
Figure 5.11


Volume change (Individual AS patients)
n = 13
Volume (mls)
55
50
45
40
35
30
25
20
15
10
5
0
Vol pre
Vol post


Volume change (Individual AS patients)
n = 14
Volume (mls)
30
20
10
0
Vol pre
Vol post


Volume change (Individual AT patients)
n = 18
Volume (mls)
45
40
35
30
25
20
15
10
5
0
Vol pre
Vol post


Volume change (Individual AT patients)
n = 17
Volume (mls)
45
40
35
30
25
20
15
10
5
0
Vol pre
Vol post


Volume change (Individual AT patients)
n = 17
Volume (mls)
60
50
40
30
20
10
0
Vol pre
Vol post

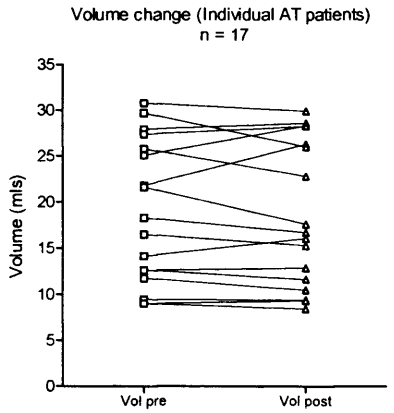

Volume change (Individual AT patients)
n = 17
Volume (mls)
35
30
25
20
15
10
5
0
Vol pre
Vol post

Figure 5.12

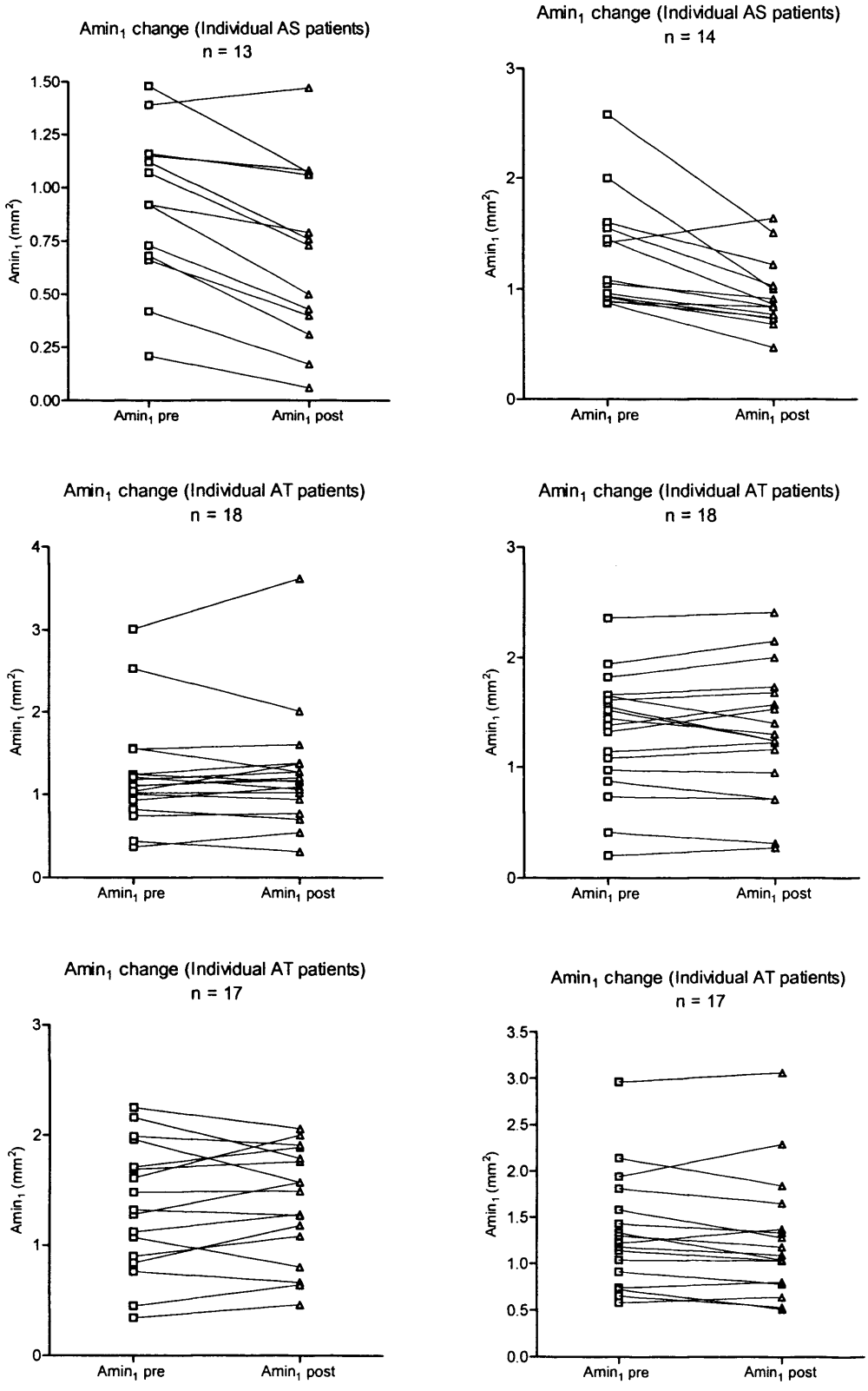

Amin1 change (Individual AS patients)
n = 13
Amin1 (mm2)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Amin1 pre
Amin1 post
Amin1 change (Individual AS patients)
n = 14
3
2
1
0
Amin1 pre
Amin1 post
Amin1 change (Individual AT patients)
n = 18
4
3
2
1
0
Amin1 pre
Amin1 post
Amin1 change (Individual AT patients)
n = 18
3
2
1
0
Amin1 pre
Amin1 post
Amin1 change (Individual AT patients)
n = 17
3
2
1
0
Amin1 pre
Amin1 post
Amin1 change (Individual AT patients)
n = 17
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Amin1 pre
Amin1 post

Figure 5.13

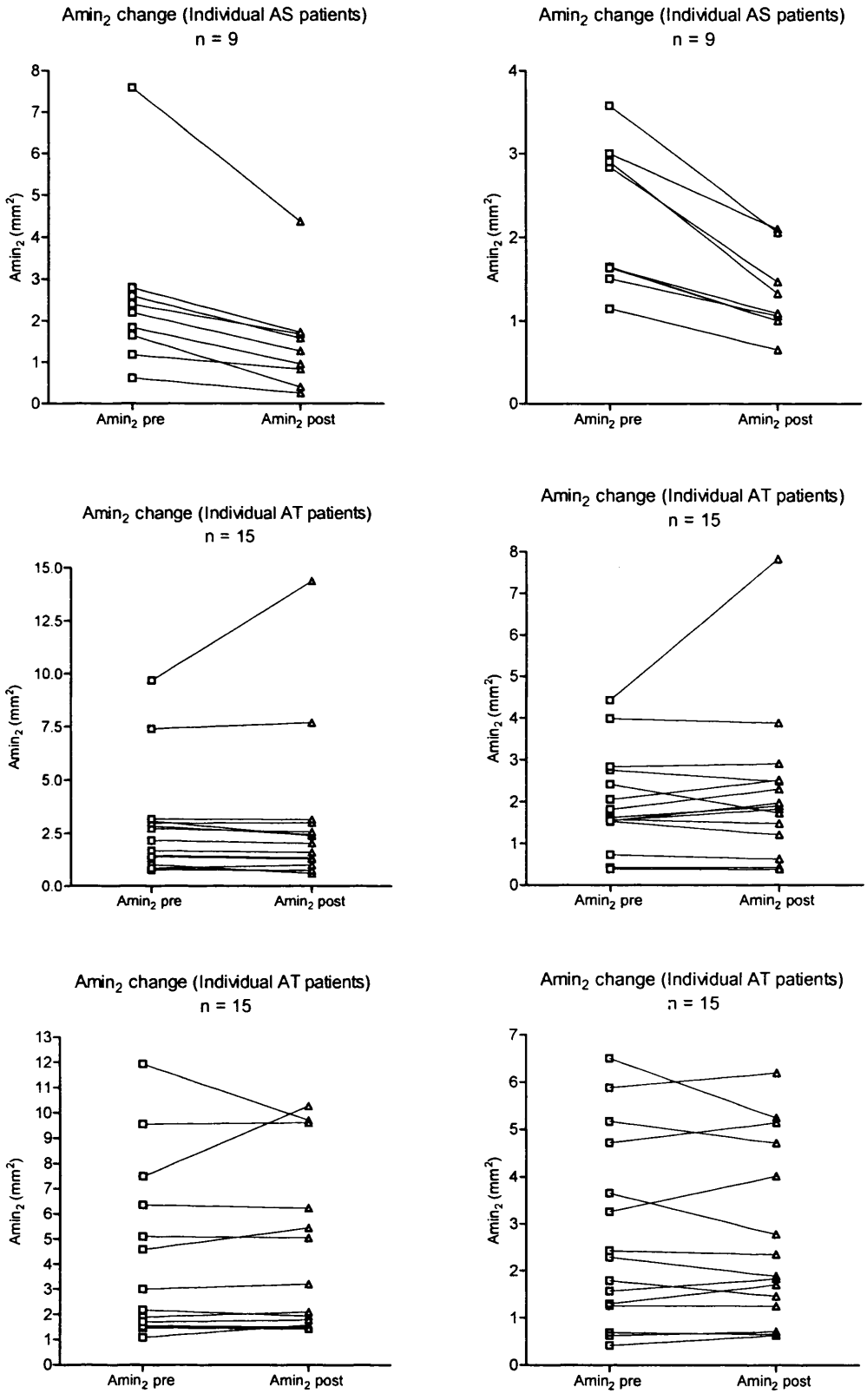

Amin2 change (Individual AS patients)
n = 9
Amin2 (mm2)
Amin2 pre
Amin2 post
Amin2 change (Individual AS patients)
n = 9
Amin2 (mm2)
Amin2 pre
Amin2 post
Amin2 change (Individual AT patients)
n = 15
Amin2 (mm2)
Amin2 pre
Amin2 post
Amin2 change (Individual AT patients)
n = 15
Amin2 (mm2)
Amin2 pre
Amin2 post
Amin2 change (Individual AT patients)
n = 15
Amin2 (mm2)
Amin2 pre
Amin2 post
Amin2 change (Individual AT patients)
n = 15
Amin2 (mm2)
Amin2 pre
Amin2 post

Figure 5.14

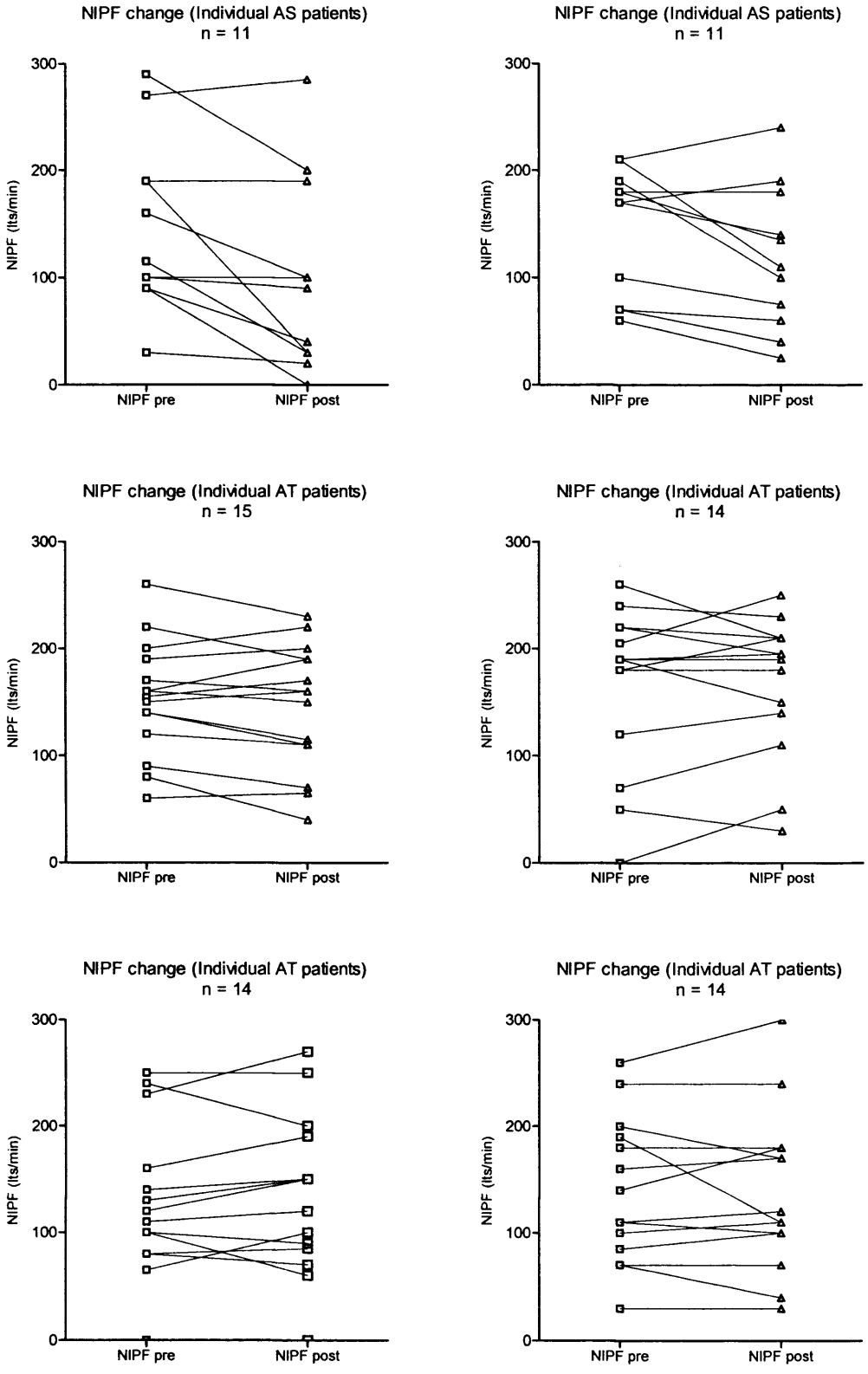

NIPF change (Individual AS patients)
n = 11
NIPF (lts/min)
300
200
100
0
NIPF pre
NIPF post
NIPF change (Individual AS patients)
n = 11
NIPF (lts/min)
300
200
100
0
NIPF pre
NIPF post
NIPF change (Individual AT patients)
n = 15
NIPF (lts/min)
300
200
100
0
NIPF pre
NIPF post
NIPF change (Individual AT patients)
n = 14
NIPF (lts/min)
300
200
100
0
NIPF pre
NIPF post
NIPF change (Individual AT patients)
n = 14
NIPF (lts/min)
300
200
100
0
NIPF pre
NIPF post
NIPF change (Individual AT patients)
n = 14
NIPF (lts/min)
300
200
100
0
NIPF pre
NIPF post

Figure 5.15

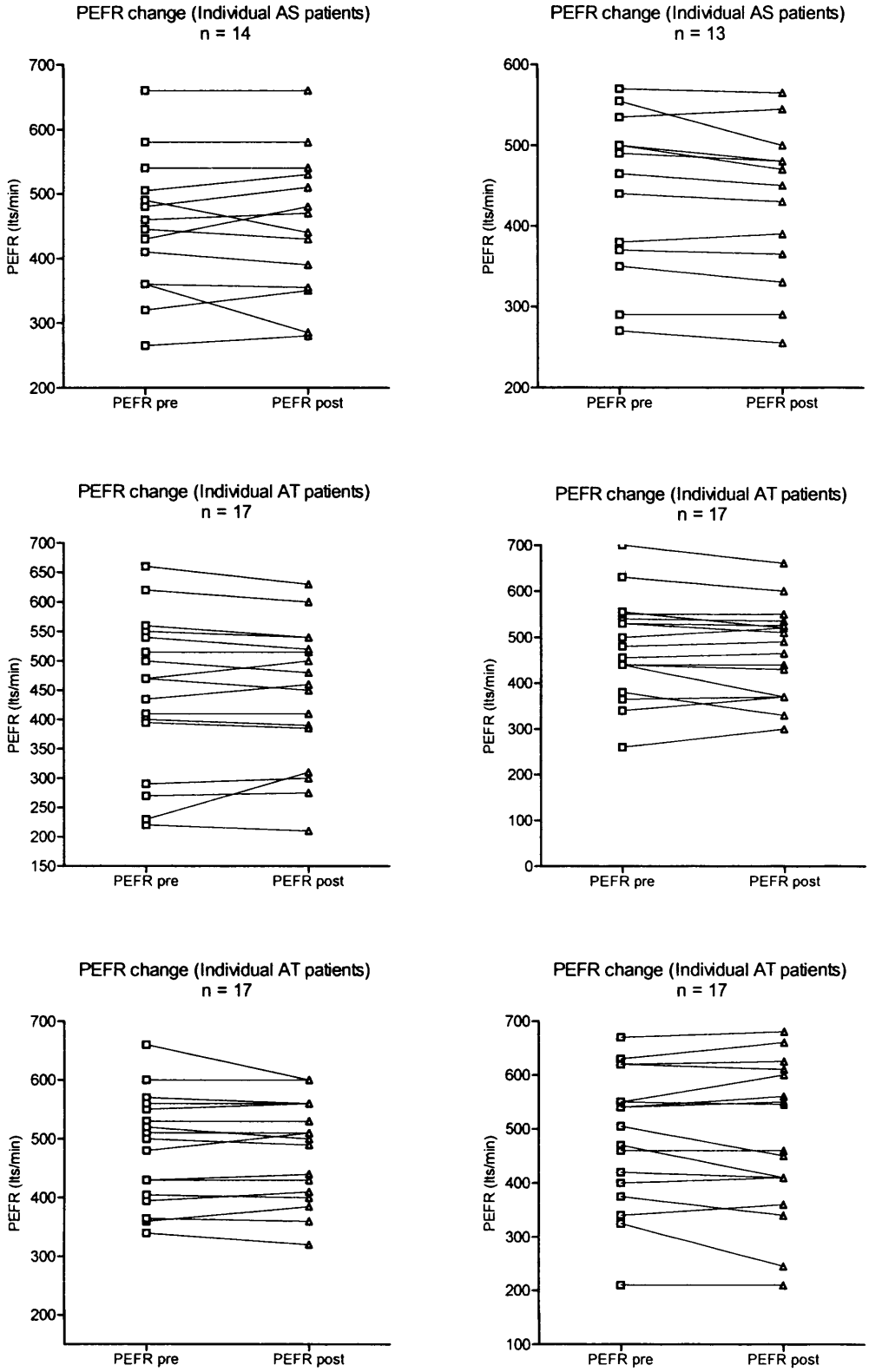
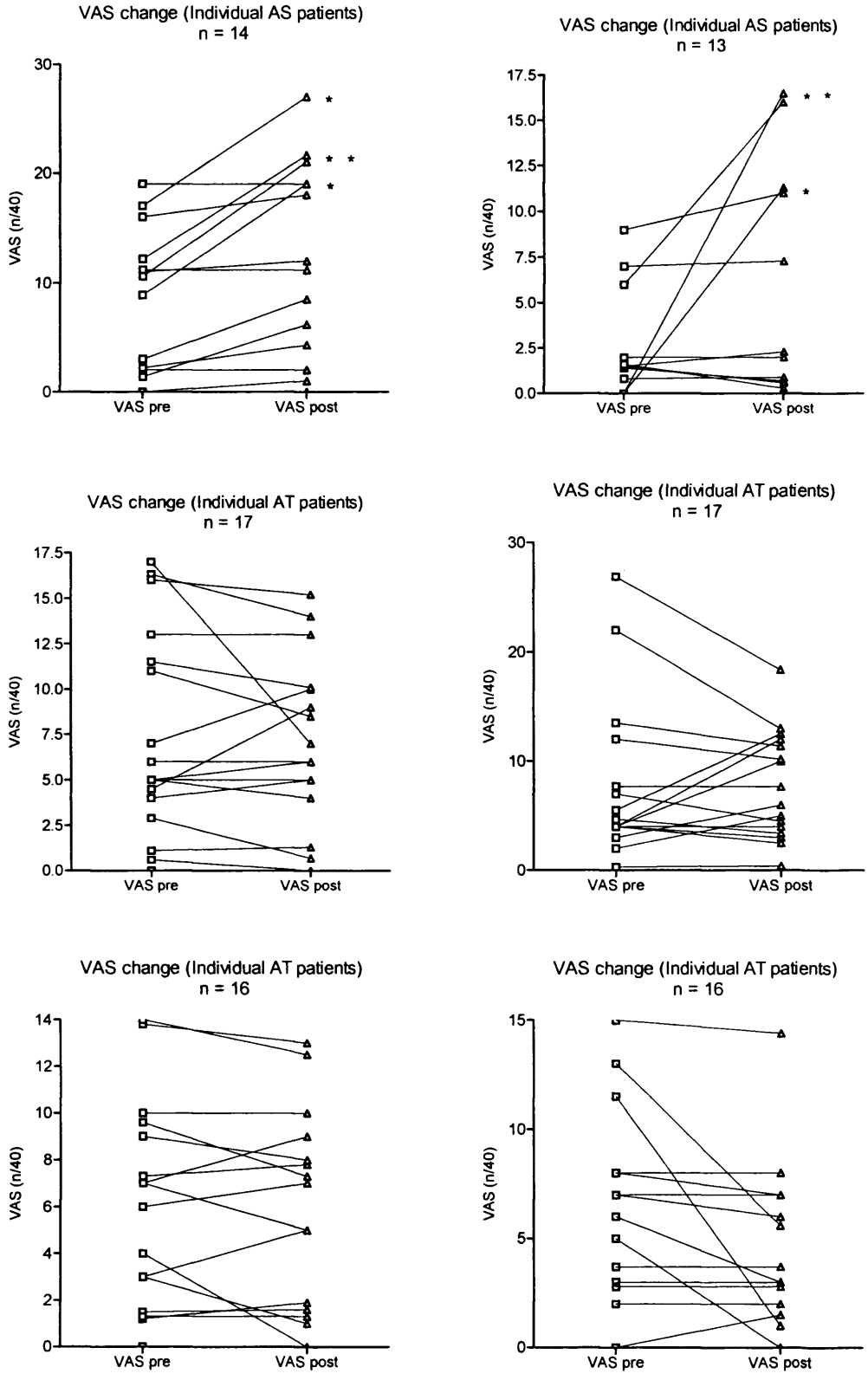

PEFR change (Individual AS patients)
n = 14
PEFR (lts/min)
700
600
500
400
300
200
PEFR pre
PEFR post
PEFR change (Individual AS patients)
n = 13
PEFR (lts/min)
600
500
400
300
200
PEFR pre
PEFR post
PEFR change (Individual AT patients)
n = 17
PEFR (lts/min)
700
650
600
550
500
450
400
350
300
250
200
150
PEFR pre
PEFR post
PEFR change (Individual AT patients)
n = 17
PEFR (lts/min)
700
600
500
400
300
200
100
0
PEFR pre
PEFR post
PEFR change (Individual AT patients)
n = 17
PEFR (lts/min)
700
600
500
400
300
200
PEFR pre
PEFR post
PEFR change (Individual AT patients)
n = 17
PEFR (lts/min)
700
600
500
400
300
200
100
PEFR pre
PEFR post

Figure 5.16 (* show change of 10 points or more)



5.1.5 *Reproducibility of Nasal challenge*

Several aspirin-sensitive patients had repeat challenges as part of their trial. Figures 5.17, 5.18, 5.19, and 5.20 show the results of patients who had 2 or more challenges. Patients who dropped out are not included as they had only 1 challenge.

The figures show that many patients had reproducible challenges. However, the degree of reaction, and the parameters that change differ with every challenge. The figures also show patients with a positive challenge and a subsequent negative challenge (BA3, RC2, YG2, SH3, LJ2, and IR2). Closer examination of the data shows that each of these challenges were after their wash-out phase. We suspect that they may still have some effect of the topical corticosteroid used in this period, and the top dose of 16 mgs of lysine-aspirin used was inadequate.

Figure 5.17

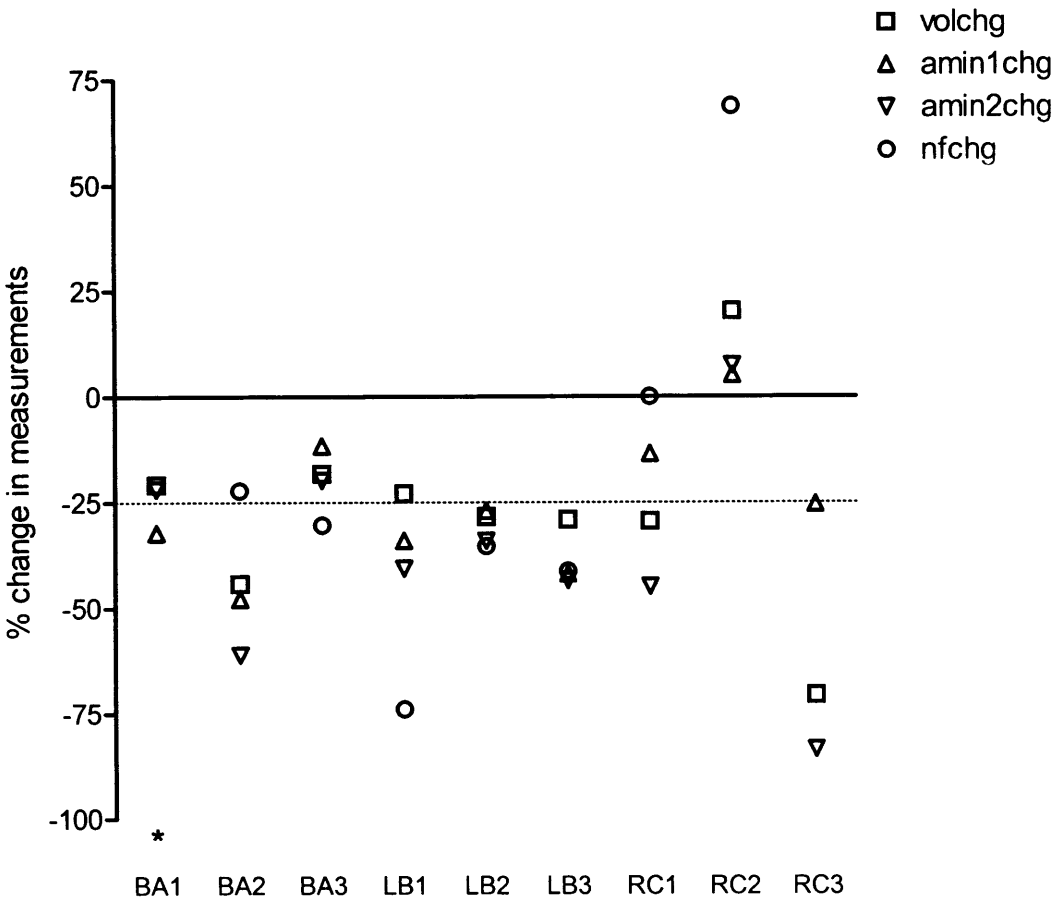

volchg
amin1chg
amin2chg
nfchg
% change in measurements
75
50
25
0
-25
-50
-75
-100
BA1
BA2
BA3
LB1
LB2
LB3
RC1
RC2
RC3

Figure 5.18

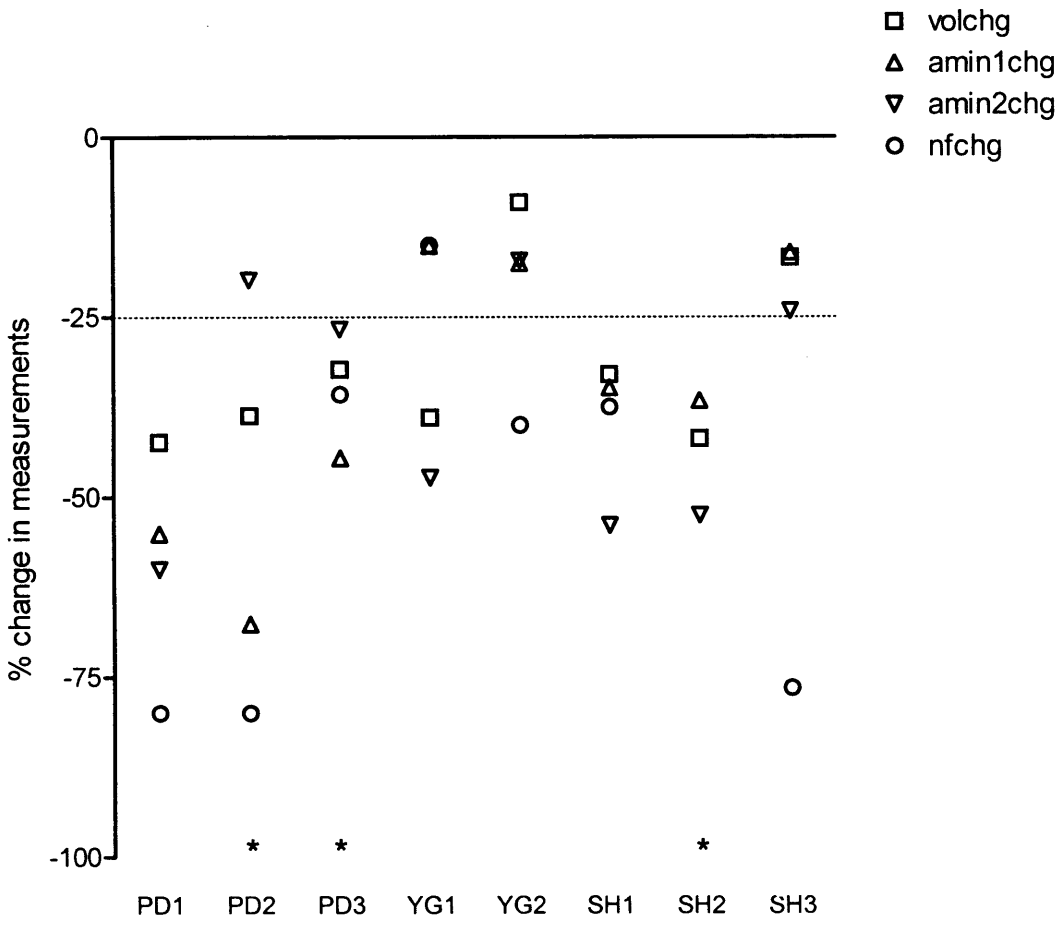

volchg
amin1chg
amin2chg
nfchg
0
-25
-50
-75
-100
% change in measurements
PD1
PD2
PD3
YG1
YG2
SH1
SH2
SH3

Figure 5.19

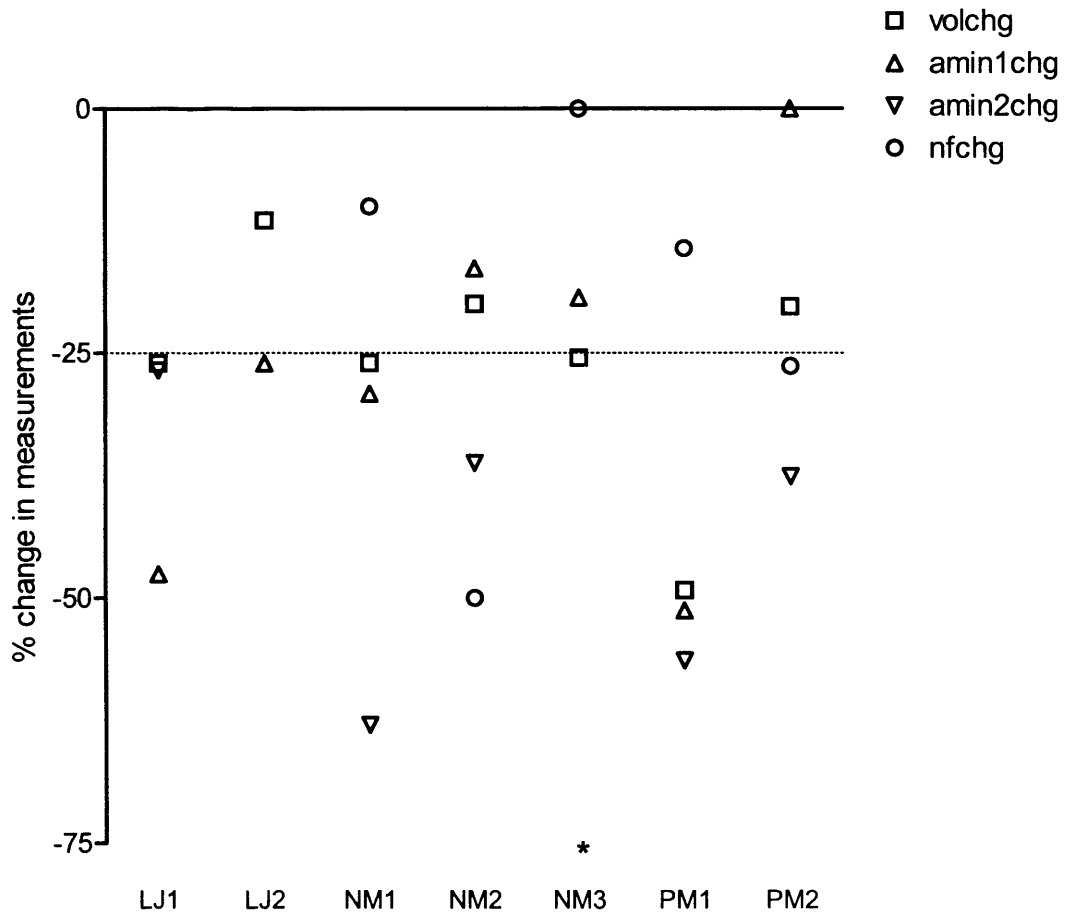
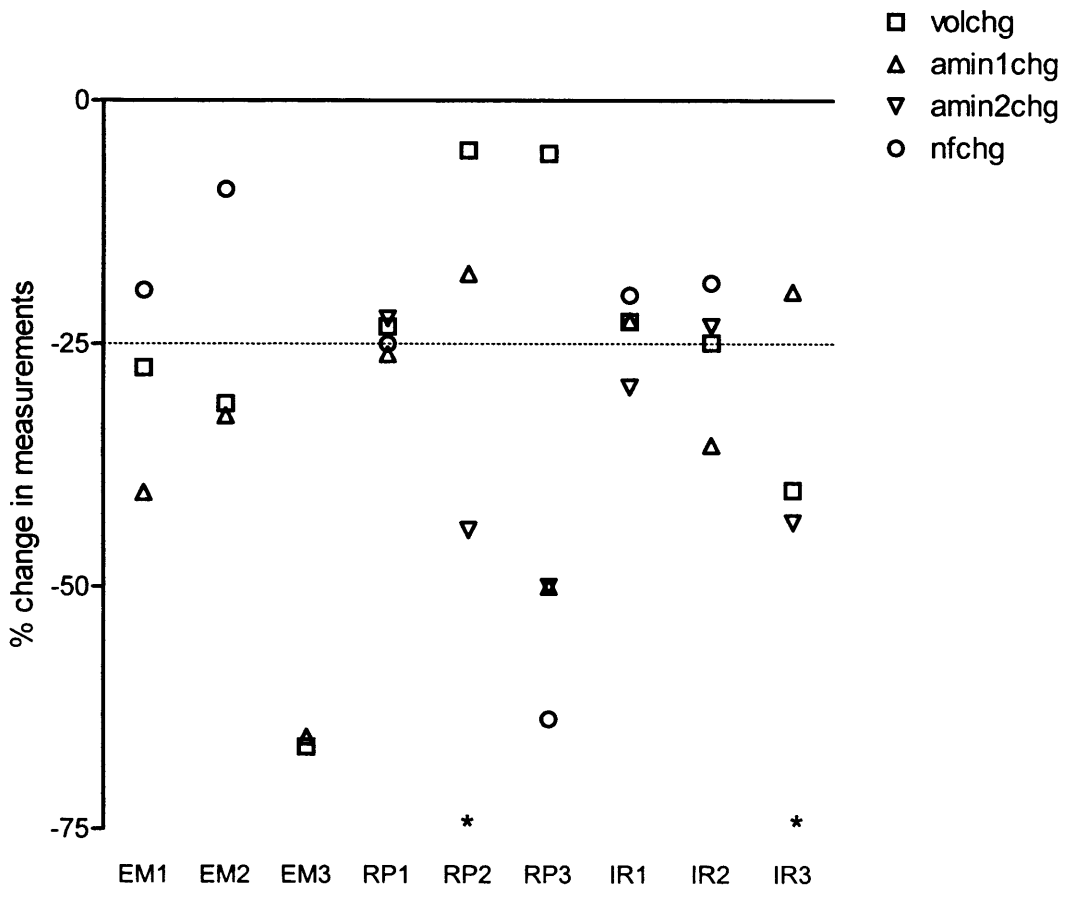

volchg
amin1chg
amin2chg
nfchg
0
-25
-50
-75
% change in measurements
LJ1
LJ2
NM1
NM2
NM3
PM1
PM2
*

Figure 5.20


volchg
amin1chg
amin2chg
nfchg
0
-25
-50
-75
% change in measurements
EM1
EM2
EM3
RP1
RP2
RP3
IR1
IR2
IR3

5.1.6 *Non-reproducible Nasal challenge*

Figure 5.21 shows 2 patients with a strong history of aspirin induced idiosyncratic reaction, but repeated negative challenges.

EC was a 67 year old lady with a 30 year history of asthma and nasal polyps. Her asthma at the time of our research was well controlled with inhalers, but in the past she had required oral steroids on 4-5 occasions. Also, she had had more than 12 polypectomies over the years. She gave a history of aspirin-induced reaction, which started within 10 minutes of taking a tablet of aspirin, but this did not need hospital treatment. Ever since the reaction she avoided all NSAIDs. Thus, we took her to be aspirin-sensitive, and entered her as in our trial of aspirin-sensitive patients. However, with repeated negative challenges we have decided to withdraw her data from analysis (see section 5.3.1).

DM was a 21 year old male with asthma and nasal polyps. He had had 5 polypectomies, and at his last admission for a routine nasal polypectomy was inadvertently given Ketorolac (NSAID). He developed severe asthma post surgery, and hence we decided to label him as aspirin-sensitive and enter him in Trial 1. He was one of our early drop-outs due to non-compliance, and hence his data has not been used for analysis (see section 5.3.1).

Figure 5.21

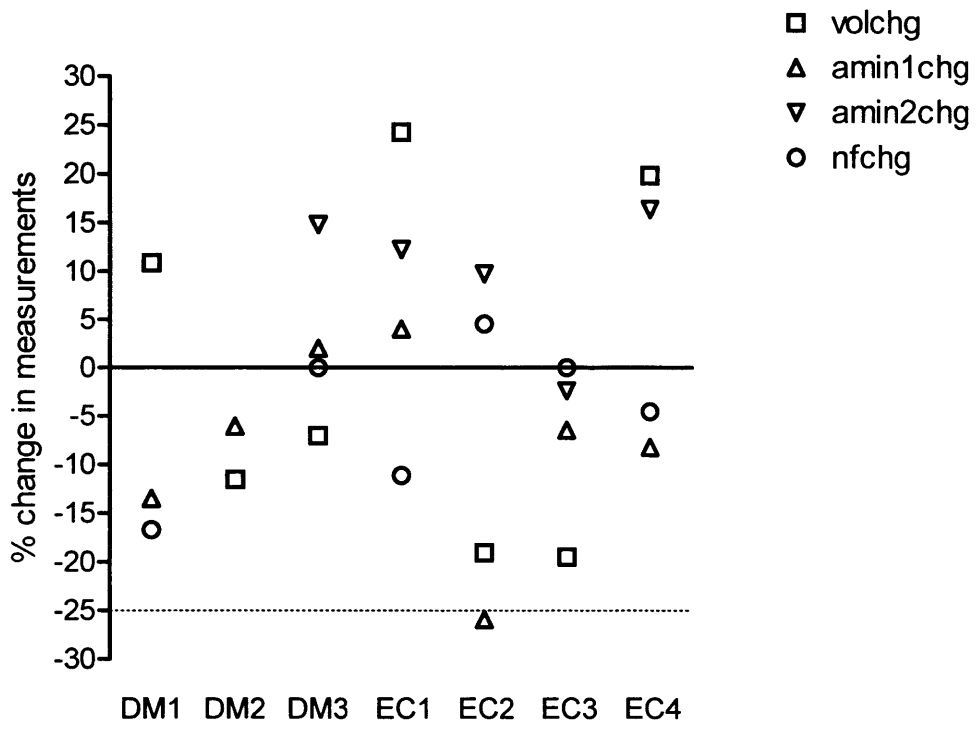
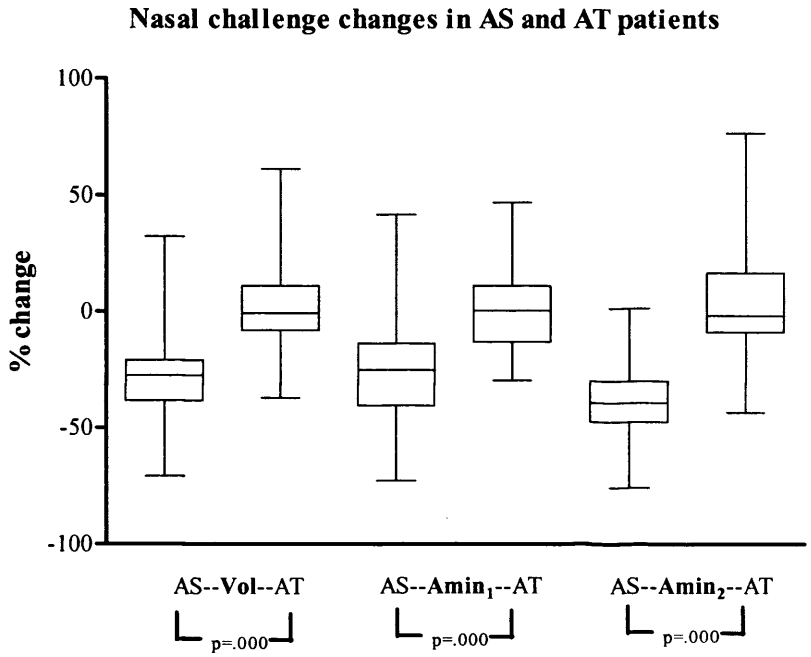
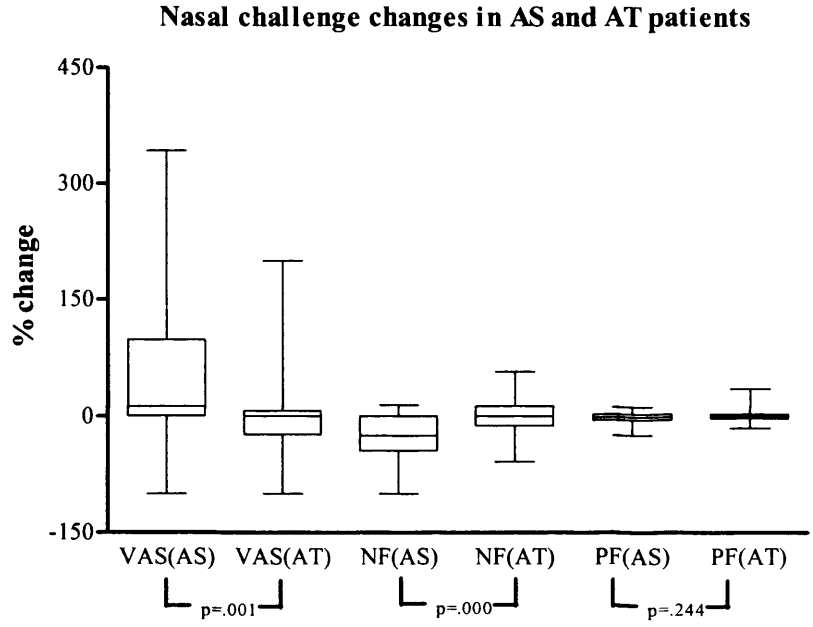

volchg
amin1chg
amin2chg
nfchg
% change in measurements
30
25
20
15
10
5
0
-5
-10
-15
-20
-25
-30
DM1
DM2
DM3
EC1
EC2
EC3
EC4

Figure 5.22 shows a comparison of changes during intranasal lysine-aspirin challenge between aspirin-sensitive and aspirin tolerant patients. Table 5.4 gives actual values.

Figure 5.22





Boxes represent 50% of values, with a line through median. Whiskers represent highest and lowest values.

Table 5.4

Changes in measured parameters following intranasal lysine-aspirin challenge – comparison of AS and AT patients

Parameter	% change		
	AS patients (n = 27)	AT patients (n = 71)	p =
	median ± S.D	median ± S.D	
Volume	-29.2 ± 15.9	-.81 ± 16.6	.000
$Amin_1$	-27.8 ± 20.2	.33 ± 17.4	.000
$Amin_2$	-40.8 ± 11.9	-.98 ± 20	.000
NIPF	-28.1 ± 31.3	0 ± 20.7	.000
PEFR	-1.4 ± 6.5	0 ± 7.4	.70

Key:

Negative values indicate deterioration.
S.D: standard deviation

5.2 Clinical characteristics

Table 5.5 compares the clinical features of the two groups classified by the results of the intranasal lysine-aspirin challenge.

Table 5.5

Comparison of AS and AT patients

Clinical feature	AS (n = 27)	AT (n = 71)	p value
Age in years: range (mean±S.D)	23-68 (38 ± 11.5)	20-69 (46 ± 10.9)	.08
Sex: Male (Female)	10 (17)	45 (26)	.02
Skin test: +ve (%)	20 (74.1)	44 (62)	.14
Asthma: Yes (%)	21 (77.8)	44 (62)	.14
History of AS: Yes (%)	22 (81.5)	14 (19.7)	.00
Surgery: Yes (%)	23 (85.2)	51 (71.8)	.17
Operations for polyps: range (mean±S.D)	0-9 (3 ± 2.9)	0-20 (1 ± 3.6)	.10
Radical surgery: Yes (%)	1 (3.7)	3 (4.2)	.13
Other surgery: Yes (%)	11 (40.7)	17 (23.9)	.15

5.3 Trial 1

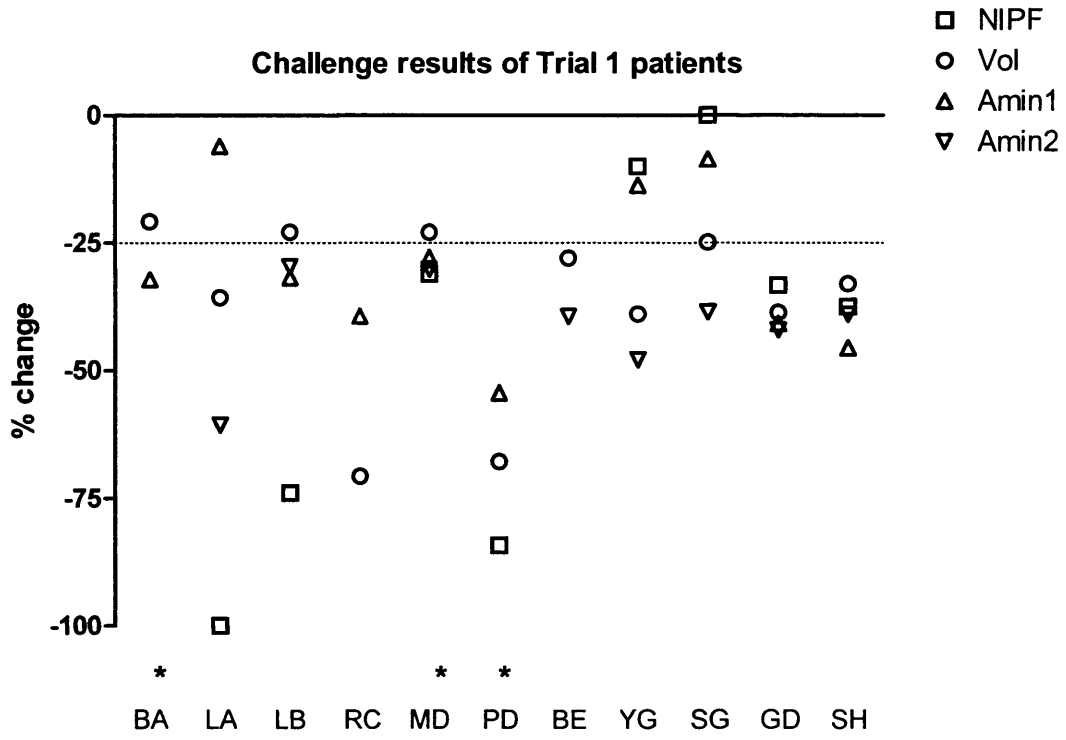
Randomised, double-blind, placebo-controlled, crossover trial to study the clinical effectiveness of intranasal lysine-aspirin in reducing nasal polyp growth, in *aspirin-sensitive* patients with nasal polyposis.

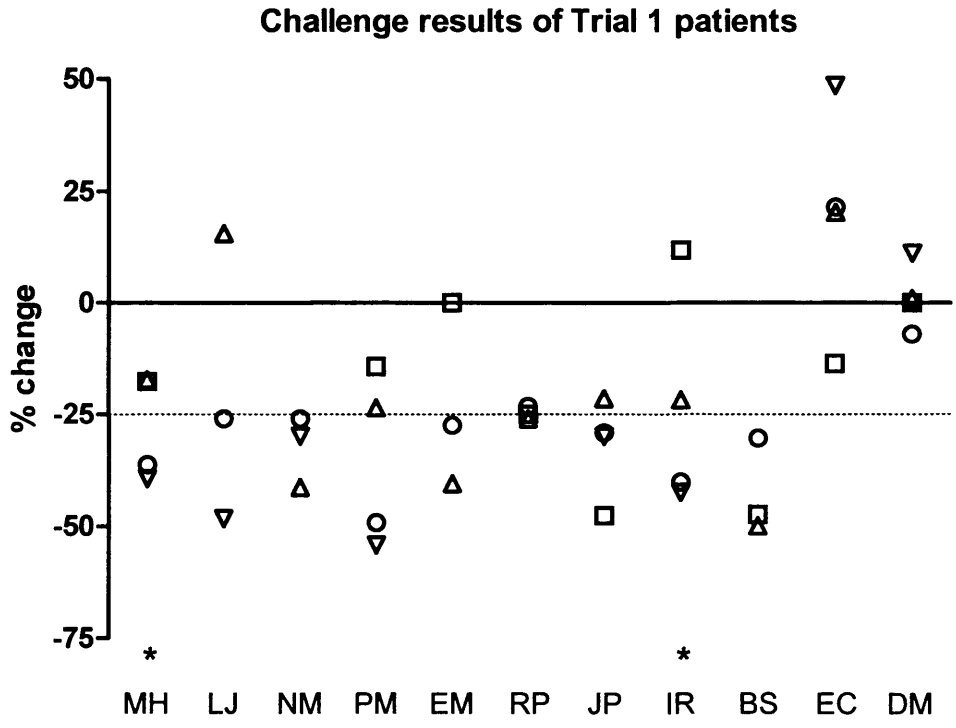
5.3.1 *Patients*

Twenty-two aspirin-sensitive patients were enrolled and randomised in this trial. These included 15 women and 7 men with an age range of 23-68 years (41.2 ± 11.9). Sixteen were skin prick test positive, 19 had a history of aspirin induced adverse reaction, and 18 had asthma. Their asthma was well controlled on inhaled corticosteroids and none of them required regular oral corticosteroids. Nineteen patients had undergone surgery for nasal polyps with the number of operations ranging from 1-20 (5.2 ± 4.5).

All 22 patients underwent intranasal lysine-aspirin challenge (Figure 5.23). Twenty patients had a positive challenge. Two patients (DM, EC) had a negative challenge (see section 5.1.5). Their data has not been included in this analysis.

Figure 5.23


Challenge results of Trial 1 patients
NIPF
Vol
Amin1
Amin2
% change
0
-25
-50
-75
-100
*
*
*
BA
LA
LB
RC
MD
PD
BE
YG
SG
GD
SH


Challenge results of Trial 1 patients
% change
50
25
0
-25
-50
-75
*
*
MH
LJ
NM
PM
EM
RP
JP
IR
BS
EC
DM

5.3.2 *Trial profile*

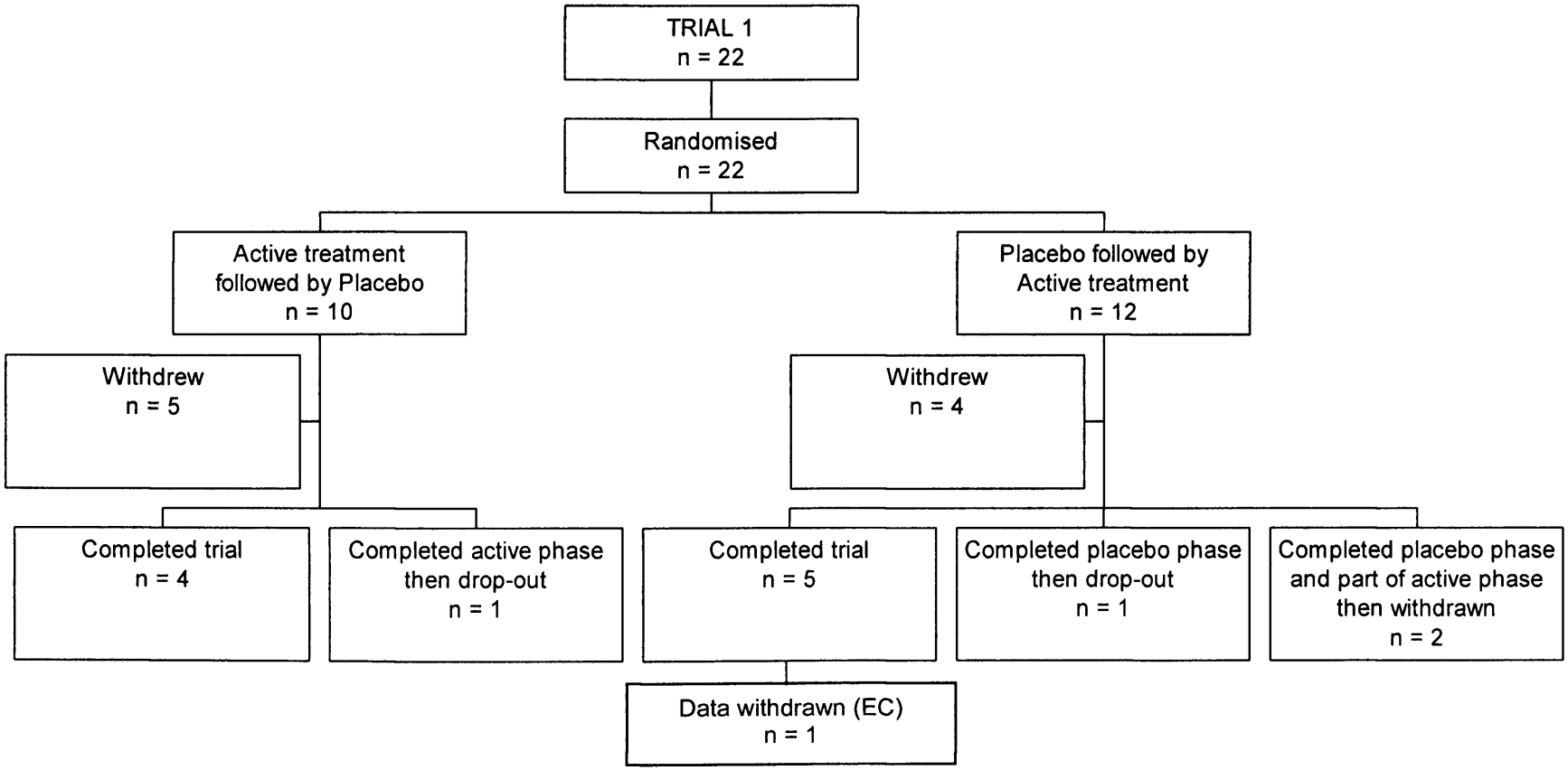
Figure 5.24 shows the trial profile. Ten patients (45.5%) were randomised to receive the active treatment (intranasal lysine-aspirin) followed by placebo, and 12 patients (54.5%) received placebo followed by the active treatment. These two arms of the trial are labelled as AP and PA respectively, and each treatment period as phase 1 or 2.

Four patients from the AP arm and 5 from the PA arm completed both phases of the trial. Two patients in the PA arm were crossed-over to the second phase but subsequently withdrawn. One of them was newly diagnosed as having multiple sclerosis, and the other patient had become non-compliant with medication. One patient in each arm completed only phase 1. Thus, we had data from both phases on 11 patients and from a single phase on 2 patients.

As mentioned in the earlier section EC completed both phases (she was in the PA arm) of the trial, but her data has been withdrawn from analysis following a revision of her aspirin-sensitive status.

Figure 5.24

Flow chart profiling Trial 1



Four patients from the AP arm and 5 from the PA arm withdrew from the trial. Two patients moved away from the area and could not follow-up; we withdrew 1 patient because he was non-compliant with the medication, and the rest withdrew because they found that the trial was more time consuming than they had expected.

5.3.3 *Statistical analysis*

Six measures were analysed. These included 2 acoustic rhinometry measures i.e. volume (0-7 cms), Amin (minimum cross-sectional area), and 4 diary card measures i.e. daily nasal and chest score, and daily nasal inspiratory and peak expiratory flow rate.

Changes in individual patients are presented in Figures 5.25 and 5.26. Comparison of data values from the start and end of each phase was done using Wilcoxon matched pairs test. Comparison of changes during each phase was done using Mann-Whitney U test. Significance level was set at $p = .05$.

Various parameters changed significantly within a phase. However, comparison of the 2 phases did not reveal any significant difference.

Figure 5.25

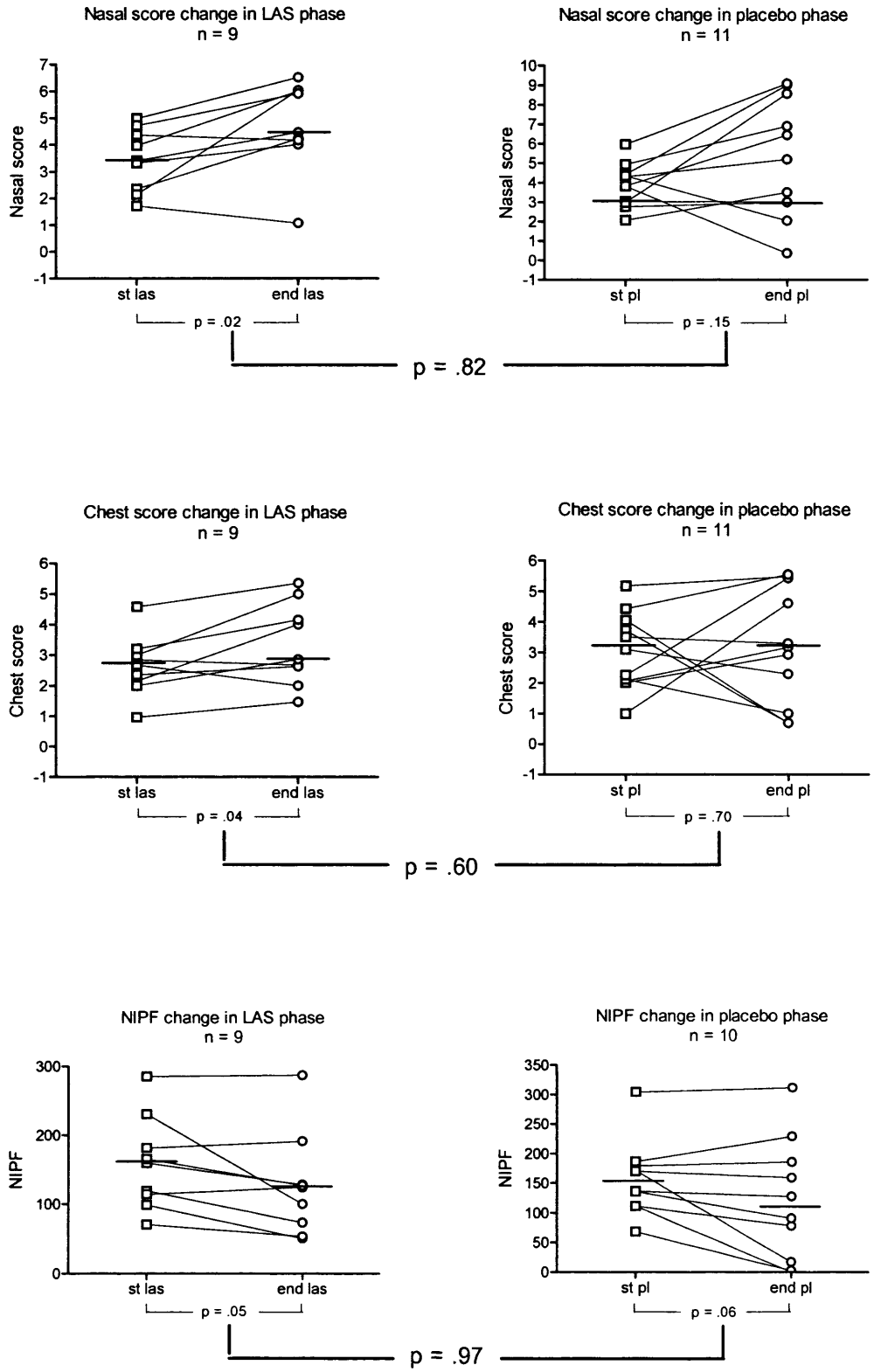
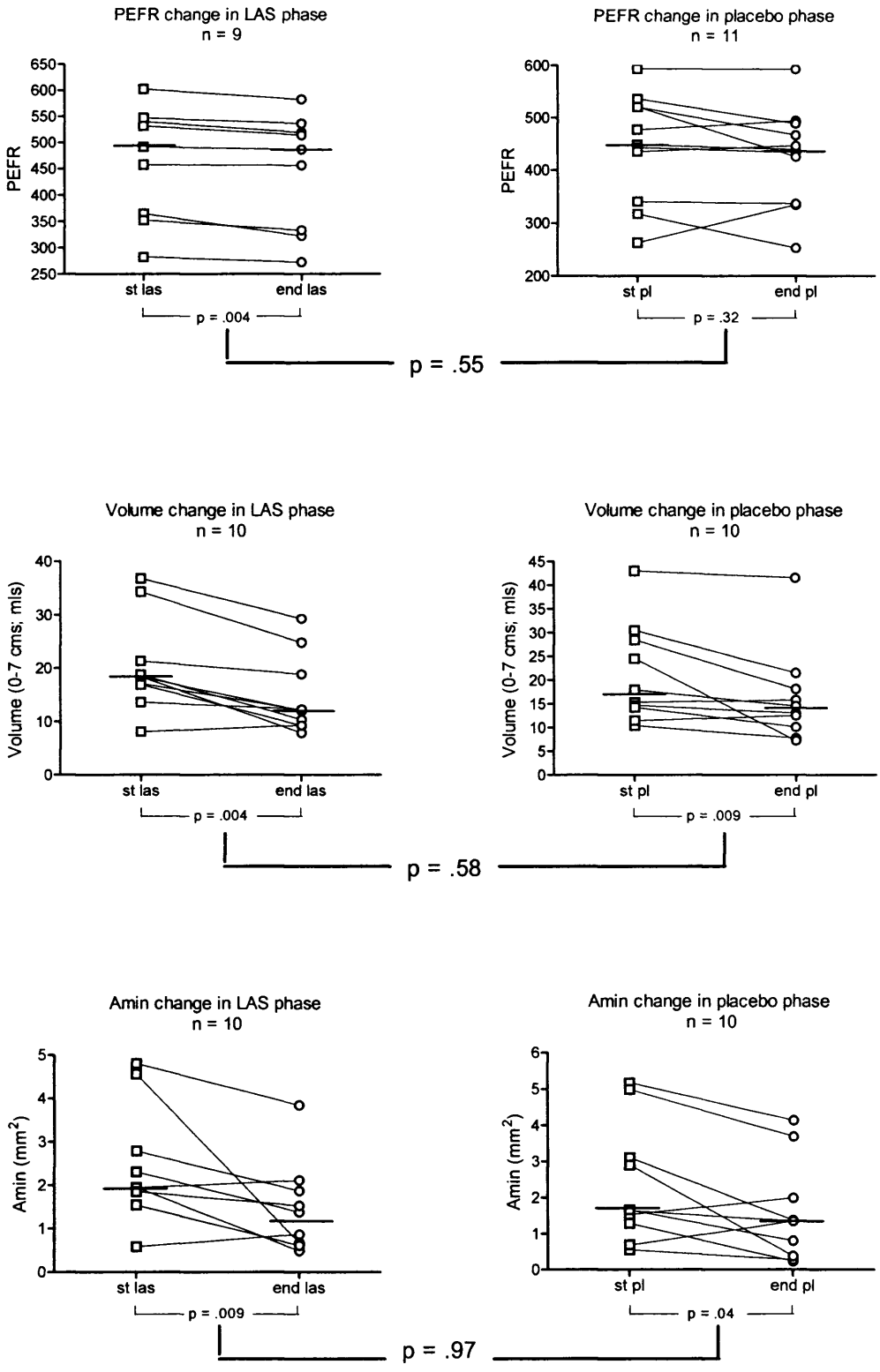

Nasal score change in LAS phase
n = 9
Nasal score
st las
end las
p = .02
Nasal score change in placebo phase
n = 11
Nasal score
st pl
end pl
p = .15
p = .82
Chest score change in LAS phase
n = 9
Chest score
st las
end las
p = .04
Chest score change in placebo phase
n = 11
Chest score
st pl
end pl
p = .70
p = .60
NIPF change in LAS phase
n = 9
NIPF
st las
end las
p = .05
NIPF change in placebo phase
n = 10
NIPF
st pl
end pl
p = .06
p = .97

Figure 5.26


PEFR change in LAS phase
n = 9
PEFR
650
600
550
500
450
400
350
300
250
st las
end las
p = .004
PEFR change in placebo phase
n = 11
600
500
400
300
200
st pl
end pl
p = .32
p = .55
Volume change in LAS phase
n = 10
Volume (0-7 cms; mls)
40
30
20
10
0
st las
end las
p = .004
Volume change in placebo phase
n = 10
45
40
35
30
25
20
15
10
5
0
st pl
end pl
p = .009
p = .58
Amin change in LAS phase
n = 10
Amin (mm2)
5
4
3
2
1
0
st las
end las
p = .009
Amin change in placebo phase
n = 10
6
5
4
3
2
1
0
st pl
end pl
p = .04
p = .97

5.3.4 *Analysis of longitudinal data*

Data from 11 patients is presented in this section. Figures 5.27–5.37 show 8 patients completing both phases, 2 patients completing one phase and withdrawing from the subsequent phase, and 1 patient completing one phase only.

i) Diary card measures: Nasal/Chest scores, NIPF, and PEFR

Trial patients were asked to maintain a daily record of their nasal and chest scores, and NIPF and PEFR on a printed diary sheet. These were averaged for each week to produce a longitudinal data set.

ii) 6-8 weekly acoustic rhinometry measurements

Trial patients were followed up every 6-8 weeks to measure their nasal airway by acoustic rhinometry.

iii) Statistical analysis

Non-parametric analysis (Mann-Whitney U test) was used to study any differences between values while a patient was in the placebo or intranasal lysine-aspirin phase. No significant difference was observed for any patient between the two phases. Significance values are displayed in the graphs. Some graphs do not have p values because Mann Whitney U test is only possible when more than 2 pairs of data are available for analysis.

Figure 5.27

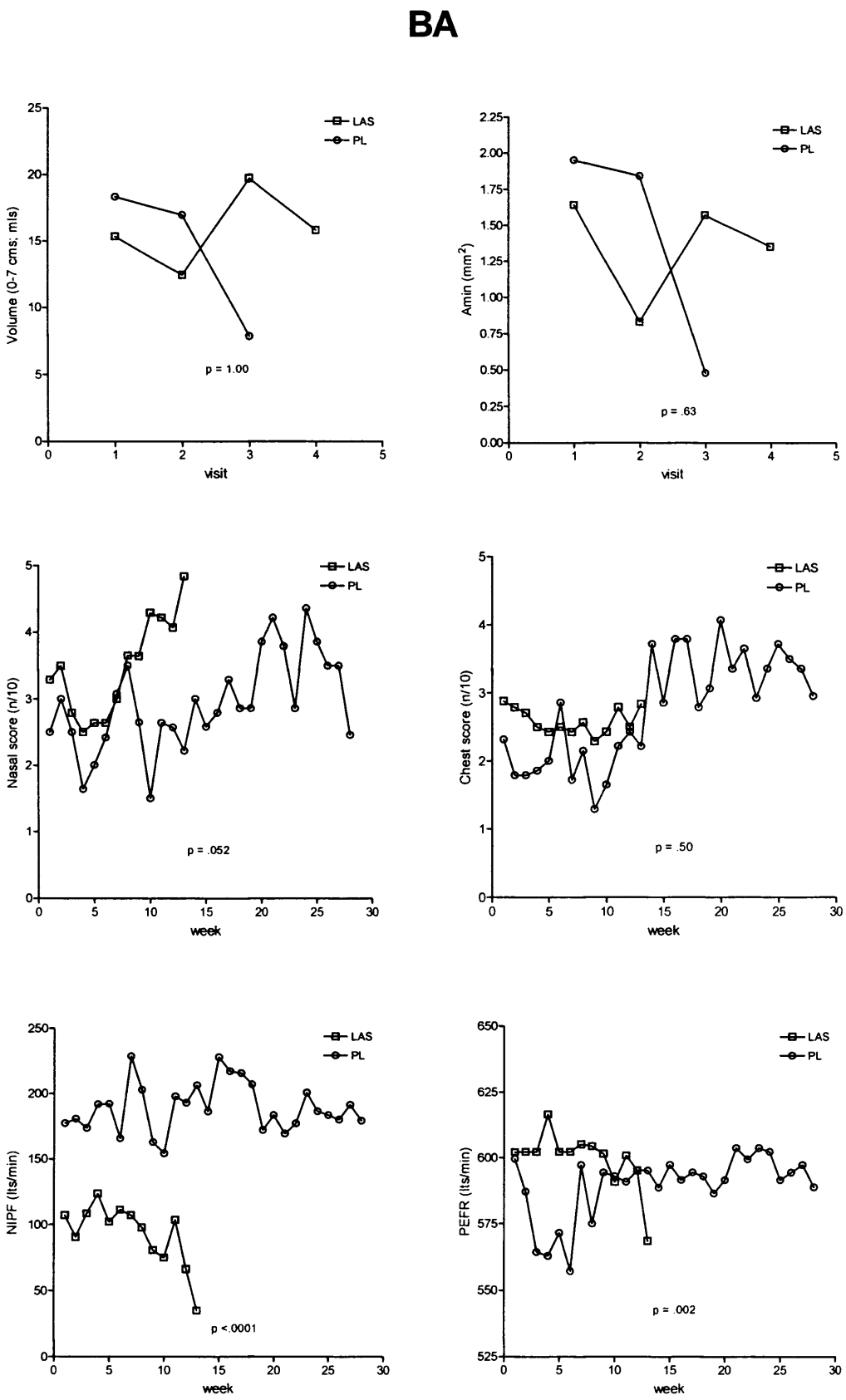

BA
LAS
PL
Volume (0-7 cms; mls)
p = 1.00
visit
Amin (mm²)
p = .63
visit
Nasal score (n/10)
p = .052
week
Chest score (n/10)
p = .50
week
NIPF (lts/min)
p <.0001
week
PEFR (lts/min)
p = .002
week

Figure 5.28

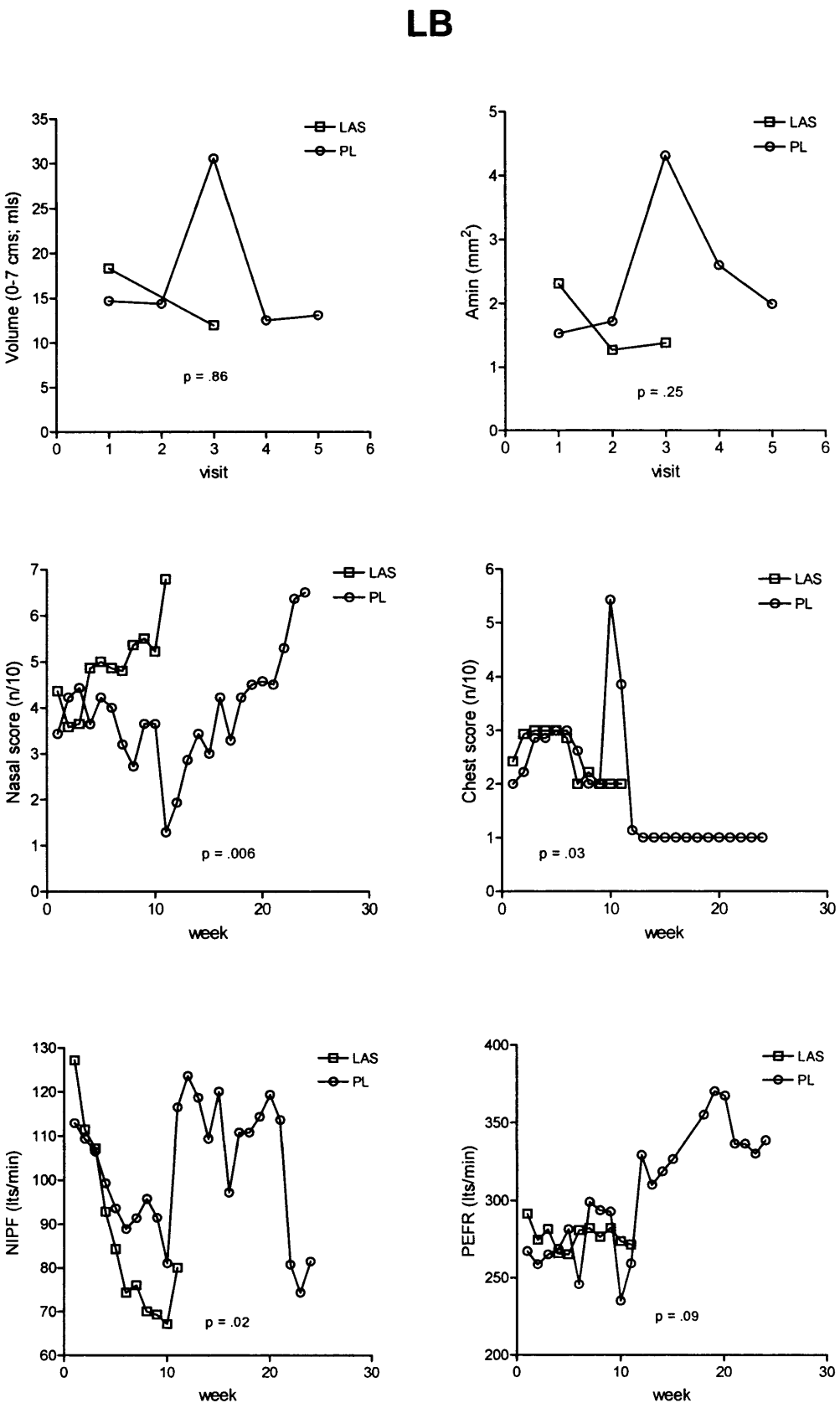

LB
LAS
PL
Volume (0-7 cms; mls)
p = .86
visit
Amin (mm²)
p = .25
visit
Nasal score (n/10)
p = .006
week
Chest score (n/10)
p = .03
week
NIPF (lts/min)
p = .02
week
PEFR (lts/min)
p = .09
week

Figure 5.29

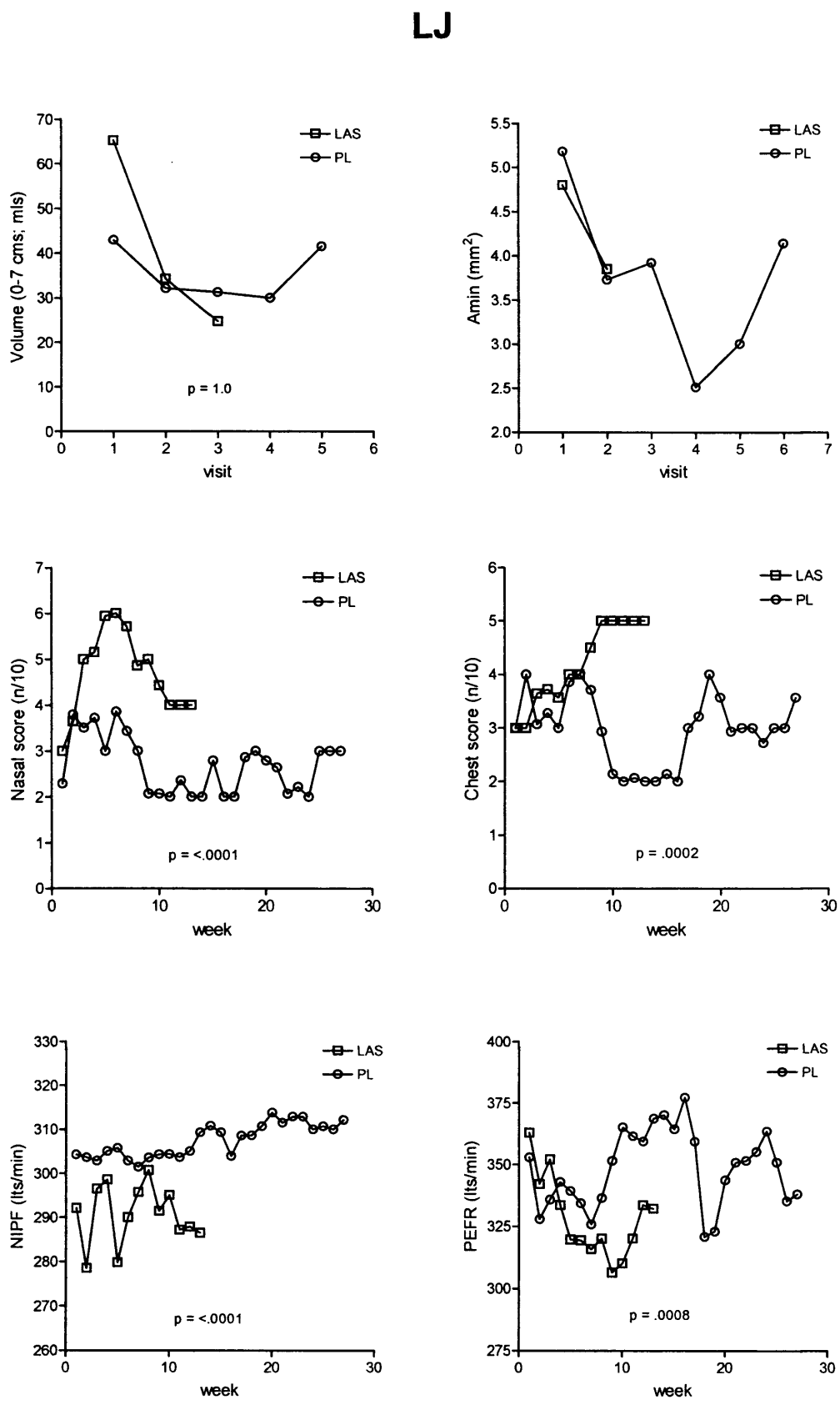

LJ
LAS
PL
Volume (0-7 cms; mls)
p = 1.0
visit
Amin (mm²)
visit
Nasal score (n/10)
p = <.0001
week
Chest score (n/10)
p = .0002
week
NIPF (lts/min)
p = <.0001
week
PEFR (lts/min)
p = .0008
week

Figure 5.30

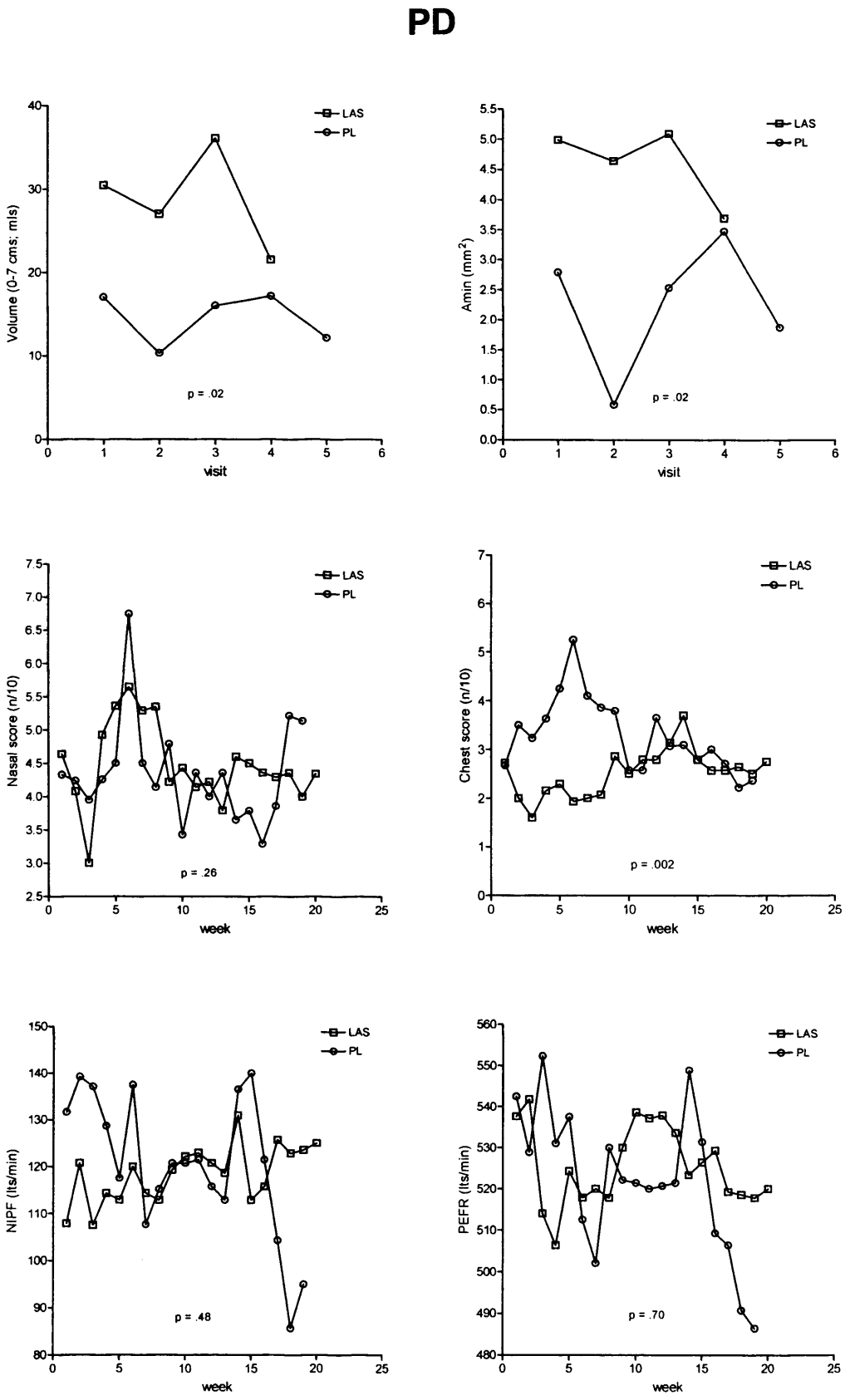

PD
LAS
PL
Volume (0-7 cms; mls)
p = .02
visit
Amin (mm²)
p = .02
visit
Nasal score (n/10)
p = .26
week
Chest score (n/10)
p = .002
week
NIPF (lts/min)
p = .48
week
PEFR (lts/min)
p = .70
week

Figure 5.31

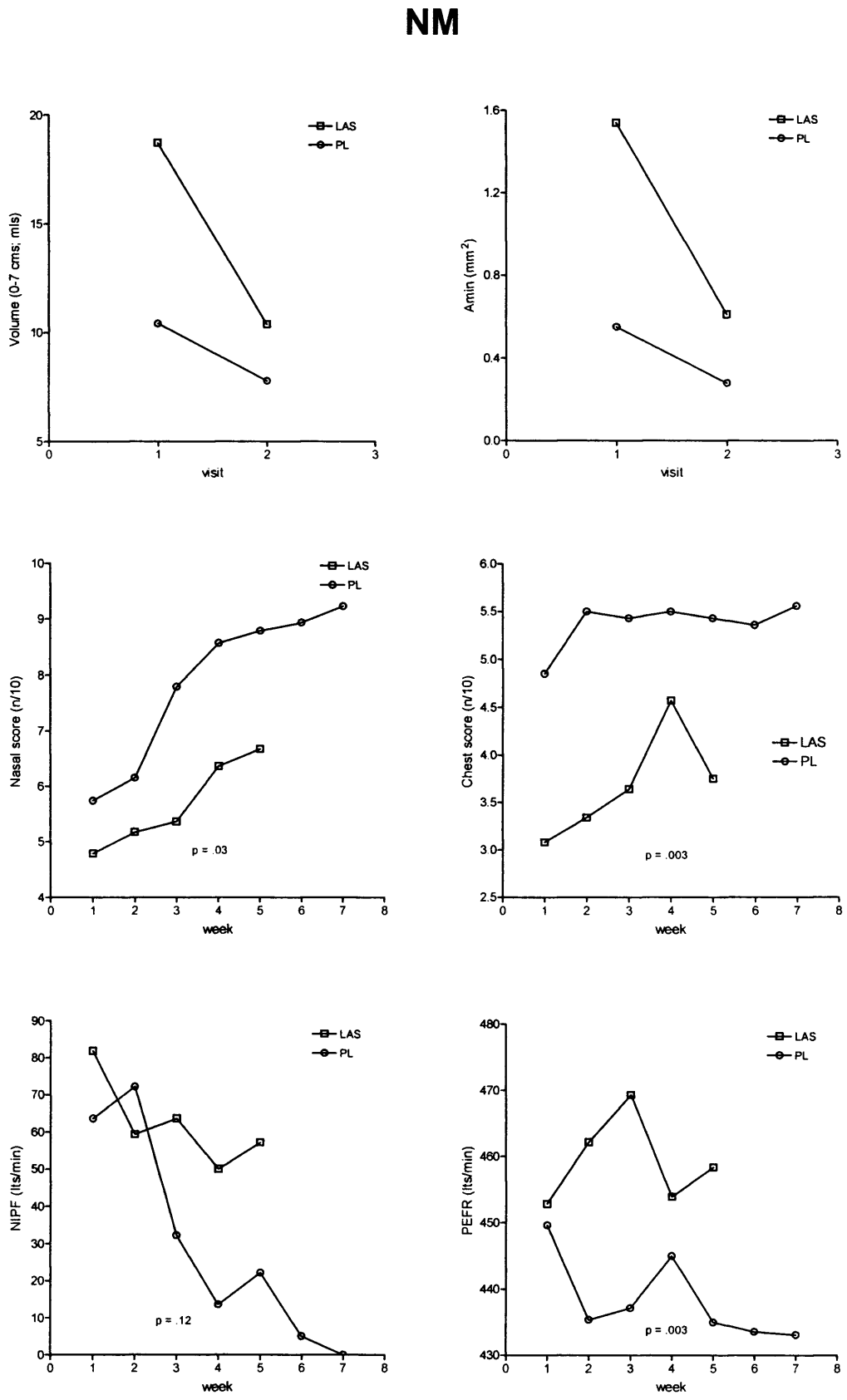

NM
Volume (0-7 cms; mls)
visit
LAS
PL
Amin (mm²)
visit
LAS
PL
Nasal score (n/10)
week
p = .03
LAS
PL
Chest score (n/10)
week
p = .003
LAS
PL
NIPF (lts/min)
week
p = .12
LAS
PL
PEFR (lts/min)
week
p = .003
LAS
PL

Figure 5.32

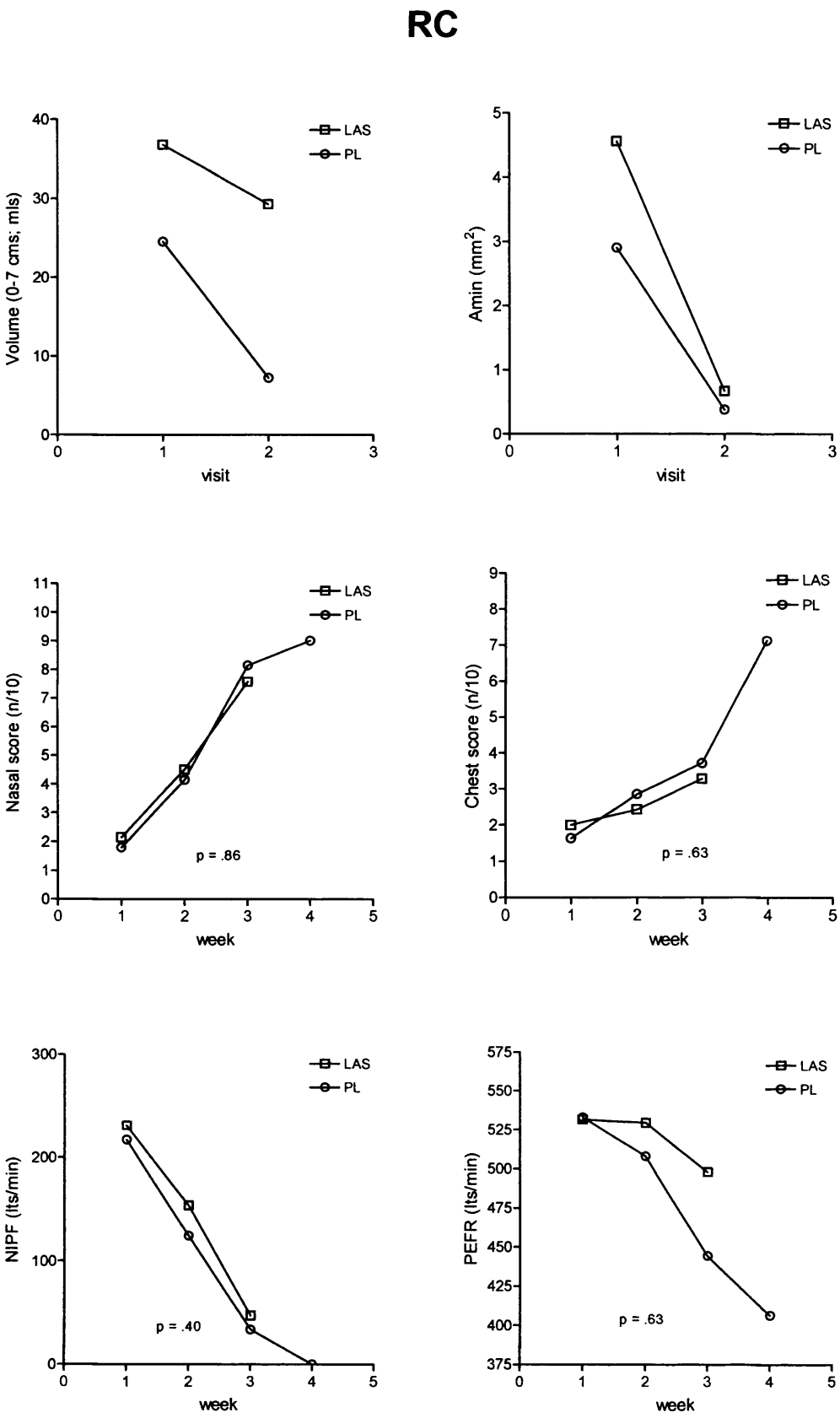

RC
Volume (0-7 cms; mls)
visit
LAS
PL
Amin (mm2)
visit
LAS
PL
Nasal score (n/10)
week
p = .86
LAS
PL
Chest score (n/10)
week
p = .63
LAS
PL
NIPF (lts/min)
week
p = .40
LAS
PL
PEFR (lts/min)
week
p = .63
LAS
PL

Figure 5.33

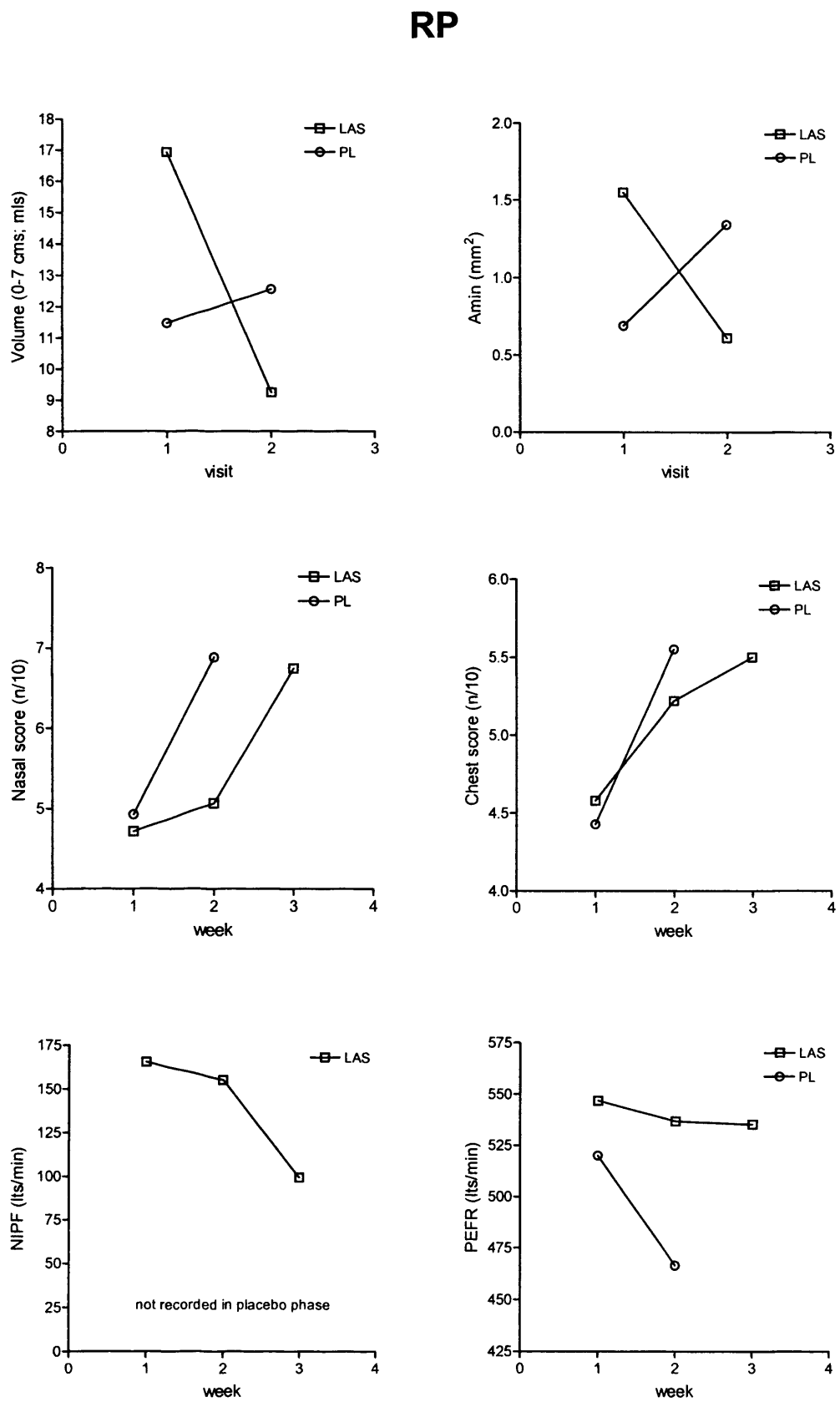

RP
LAS
PL
Volume (0-7 cms; mls)
visit
Amin (mm²)
visit
Nasal score (n/10)
week
Chest score (n/10)
week
NIPF (lts/min)
not recorded in placebo phase
week
PEFR (lts/min)
week

Figure 5.34

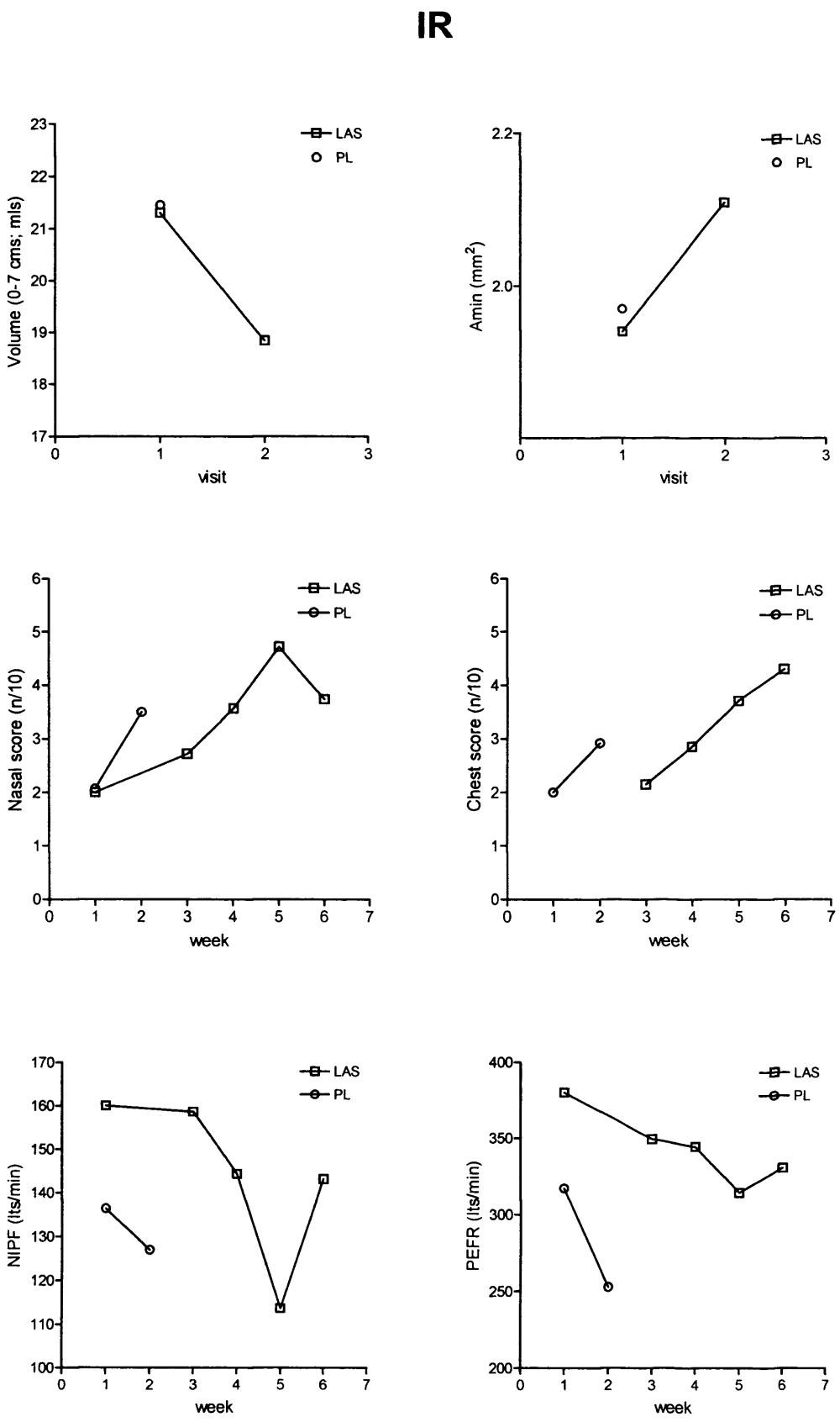

IR
LAS
PL
Volume (0-7 cms; mls)
visit
Amin (mm²)
visit
Nasal score (n/10)
week
Chest score (n/10)
week
NIPF (lts/min)
week
PEFR (lts/min)
week

Figure 5.35

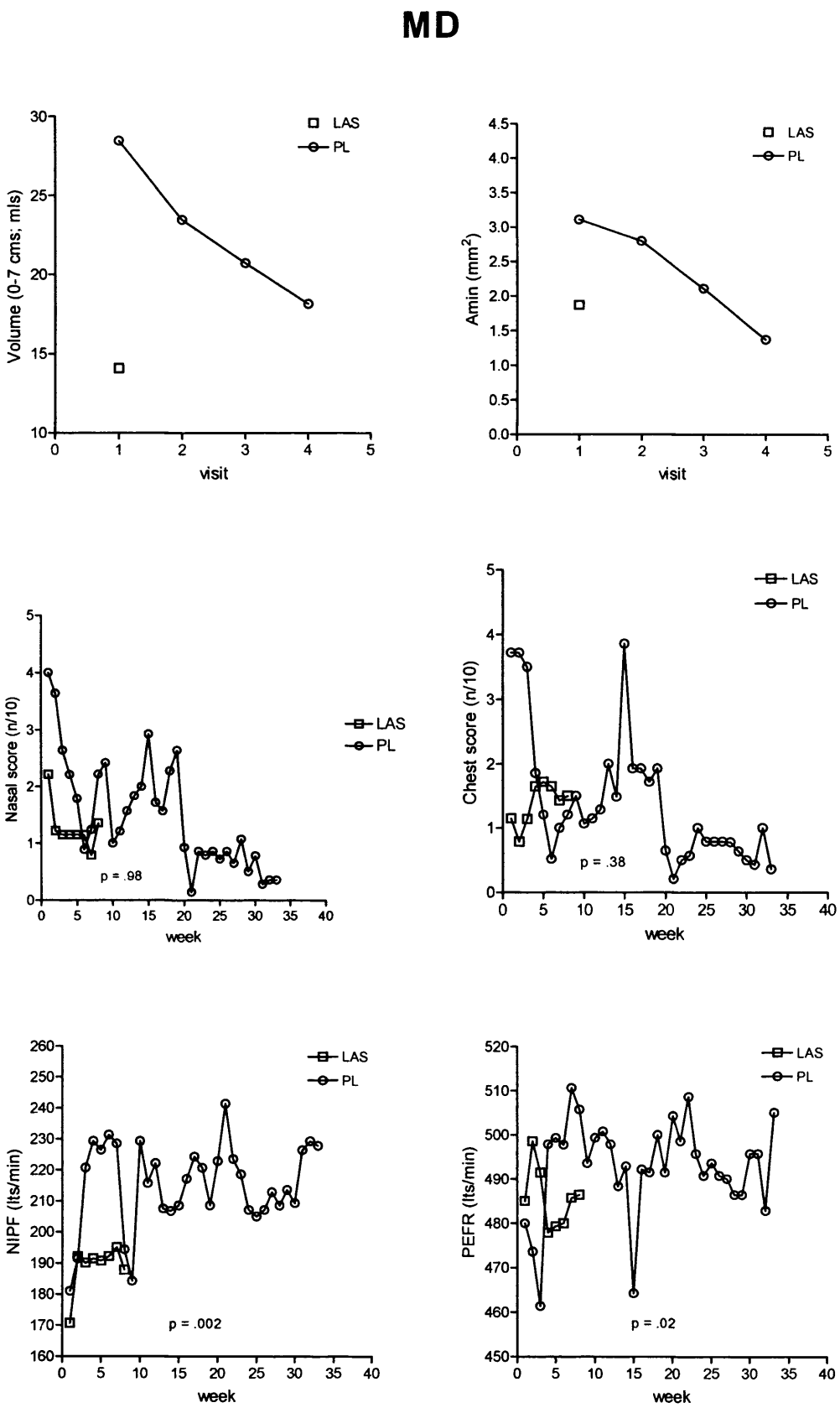

MD
LAS
PL
Volume (0-7 cms; mls)
visit
Amin (mm2)
visit
Nasal score (n/10)
p = .98
week
Chest score (n/10)
p = .38
week
NIPF (lts/min)
p = .002
week
PEFR (lts/min)
p = .02
week

Figure 5.36

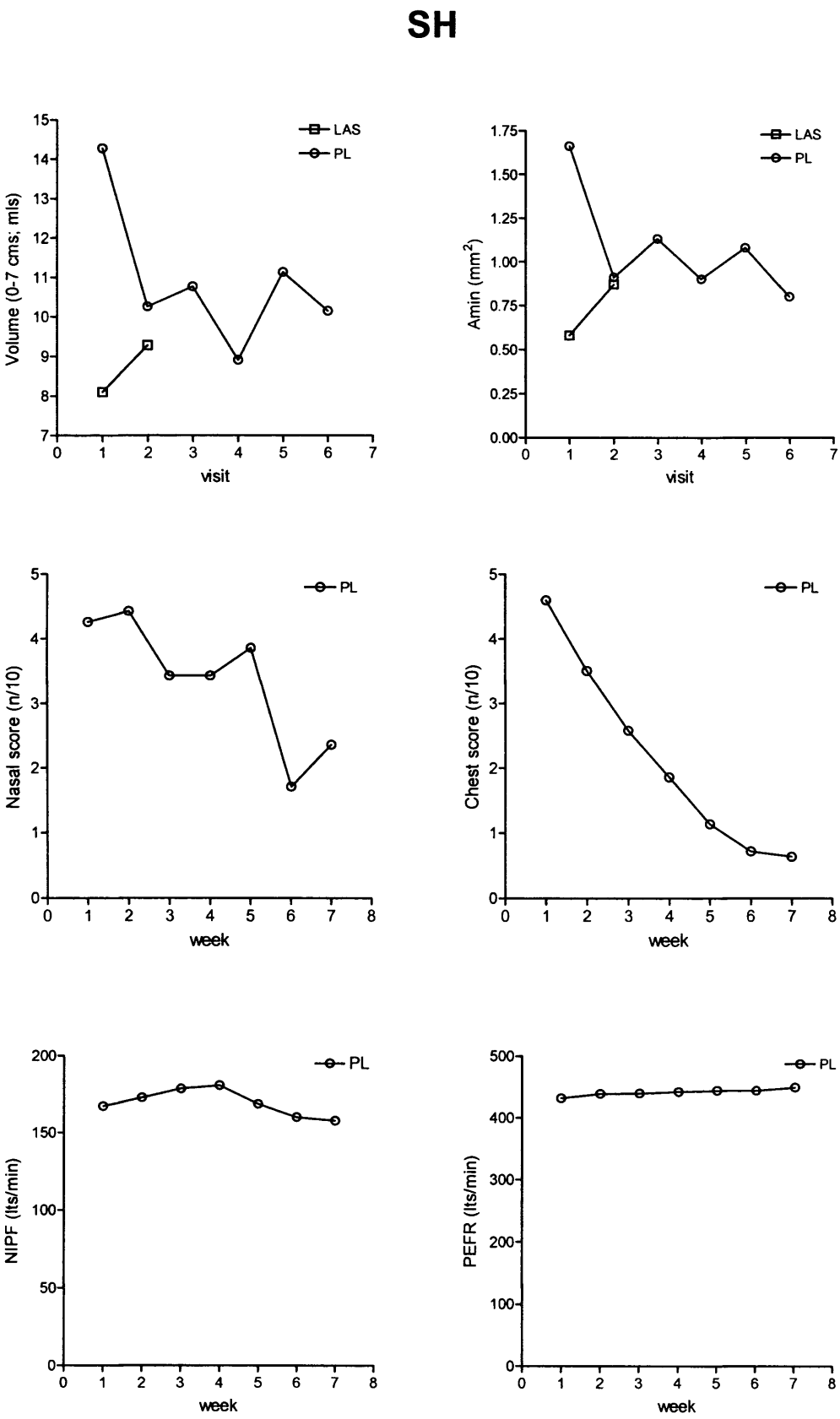
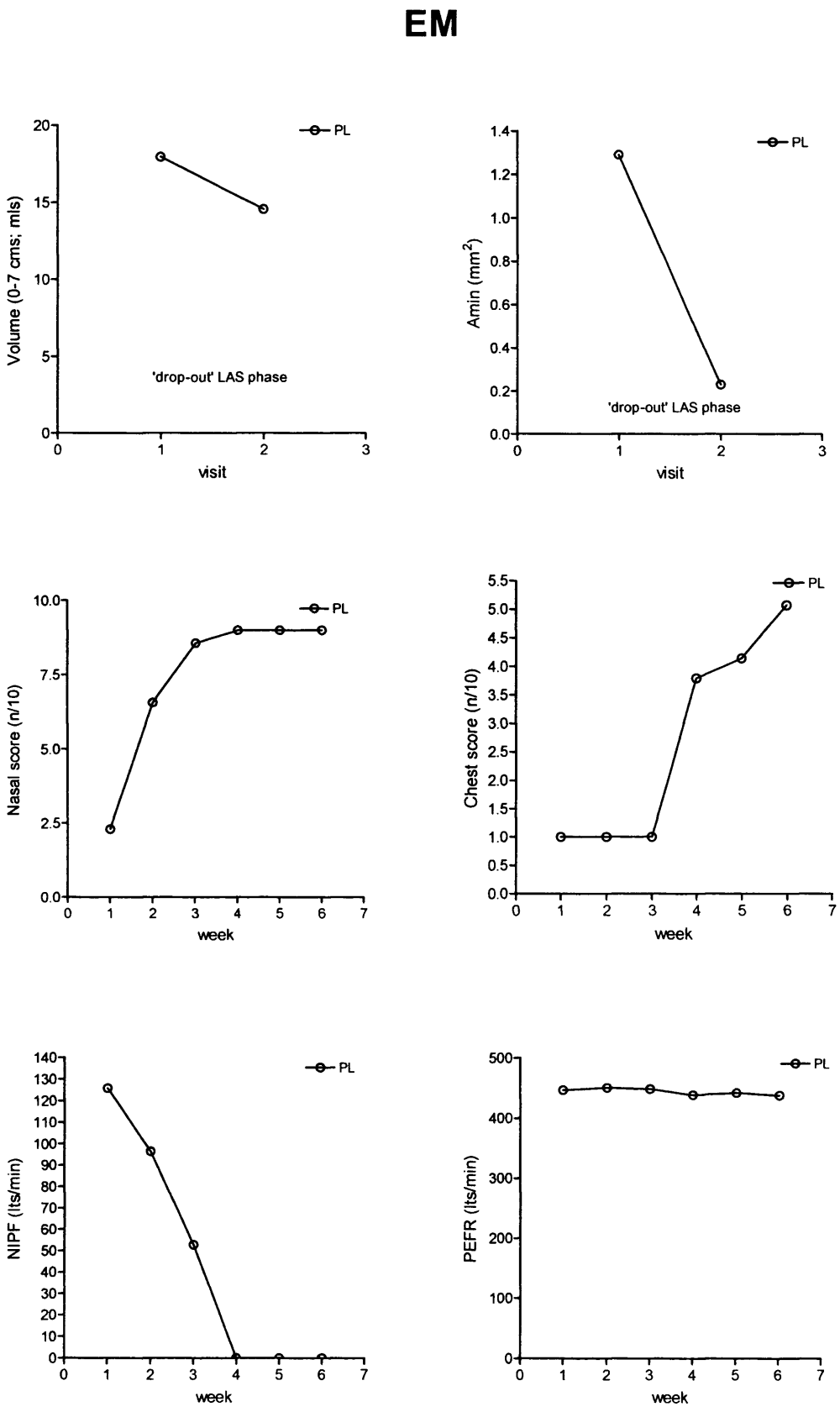

SH
LAS
PL
Volume (0-7 cms; mls)
visit
Amin (mm2)
visit
Nasal score (n/10)
week
Chest score (n/10)
week
NIPF (lts/min)
week
PEFR (lts/min)
week

Figure 5.37


EM
PL
Volume (0-7 cms; mls)
'drop-out' LAS phase
visit
Amin (mm2)
'drop-out' LAS phase
visit
Nasal score (n/10)
week
Chest score (n/10)
week
NIPF (lts/min)
week
PEFR (lts/min)
week

5.4 Trial 2

Randomised, double-blind, placebo-controlled, parallel group trial to study the clinical effectiveness of intranasal lysine-aspirin in conjunction with intranasal corticosteroid in reducing nasal polyp growth, in *aspirin-tolerant* patients with nasal polyposis.

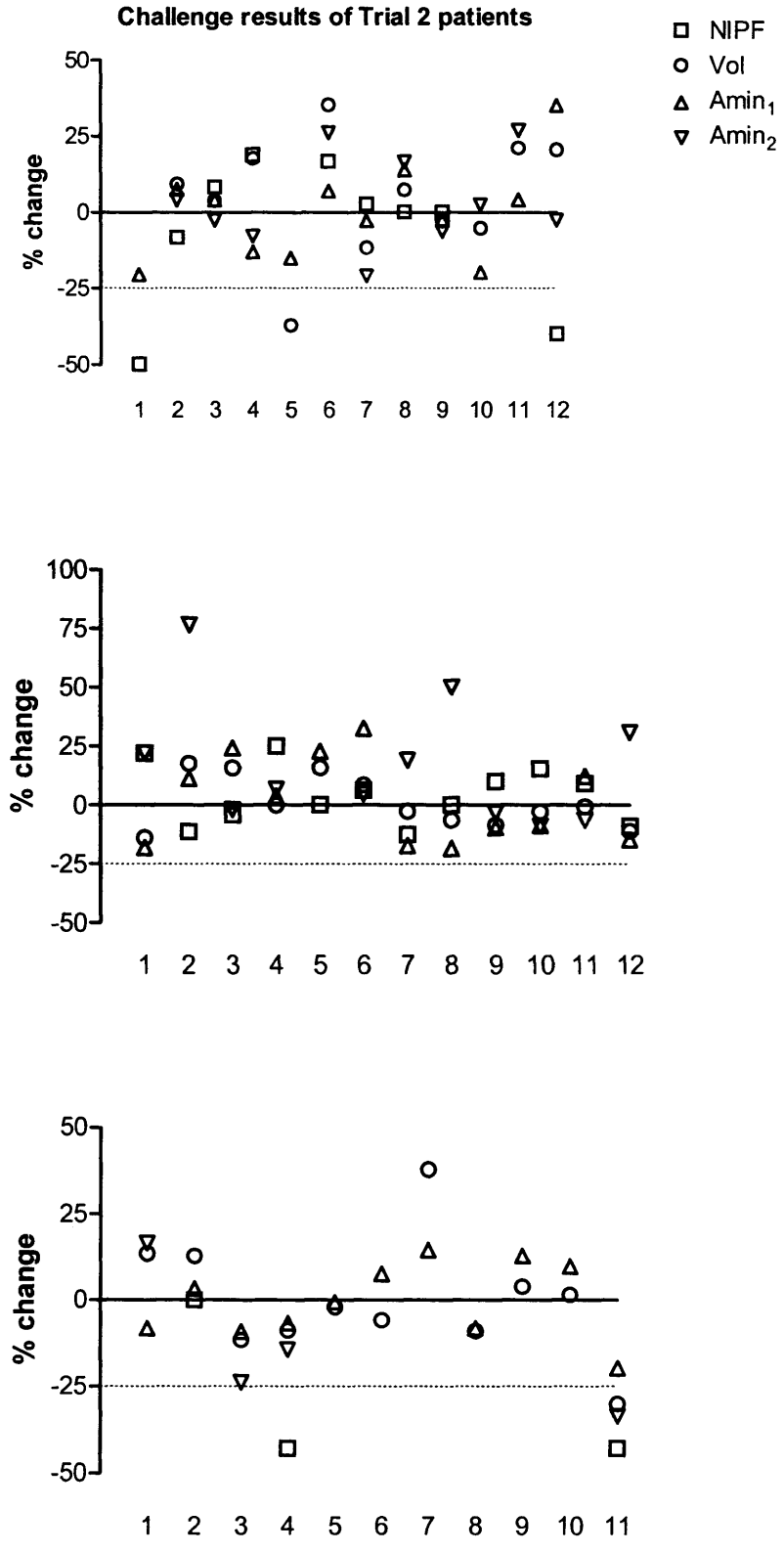
5.4.1 *Patients*

Forty aspirin tolerant patients were enrolled and randomised in this trial. These included 12 women and 28 men with an age range of 28-73 years (47.1 ± 10.6). Twenty-three were skin prick test positive, 39 could tolerate aspirin, 1 was unsure about an aspirin/NSAID induced adverse reaction, and 21 had asthma. Their asthma was well controlled on inhaled corticosteroids and none of them required regular oral corticosteroids. Thirty-four patients had undergone surgery for nasal polyps with the number of operations ranging from 1-16 (3.3 ± 3.6).

Thirty-five of 40 patients had an intranasal lysine-aspirin challenge (Figure 5.38). Of these 34 had a negative and 1 had a positive challenge (no. 11 on bottom graph). Of the 5 patients who did not have an intranasal lysine-aspirin challenge, 3 had taken aspirin/NSAID in the recent past without any adverse reaction, and 2 patients were on regular low dose aspirin.

The patient with a positive challenge was on regular aspirin and hence we decided to include her in this aspirin tolerant group as we did the other 2 patients on regular aspirin. However, with hindsight we realize that they do not qualify as aspirin tolerant, and hence have removed them from the analysis of this trial. Thus, on final count we had 37 patients in this trial.

Figure 5.38


Challenge results of Trial 2 patients
NIPF
Vol
Amin1
Amin2
% change
50
25
0
-25
-50
1 2 3 4 5 6 7 8 9 10 11 12
% change
100
75
50
25
0
-25
-50
1 2 3 4 5 6 7 8 9 10 11 12
% change
50
25
0
-25
-50
1 2 3 4 5 6 7 8 9 10 11

5.4.2 *Trial profile*

Figure 5.39 shows the trial profile. Twenty patients were randomised into each arm of the trial. Four patients from Group A did not complete the trial, as they were lost to follow-up. Three patients did not come back after the enrolment visit and 1 did not attend after 2 visits. In Group B, 1 patient was withdrawn as her asthma deteriorated. Thus, 35 patients (active group: 16; placebo group: 19) completed the trial with a follow up of 3-33 months (mean ± S.D: 14.3 ± 9.4). The follow up periods for both groups matched (Table 5.6), without any statistical difference. Further comparison of the groups is shown in Table 5.6.

Figure 5.39

Profile of Trial 2

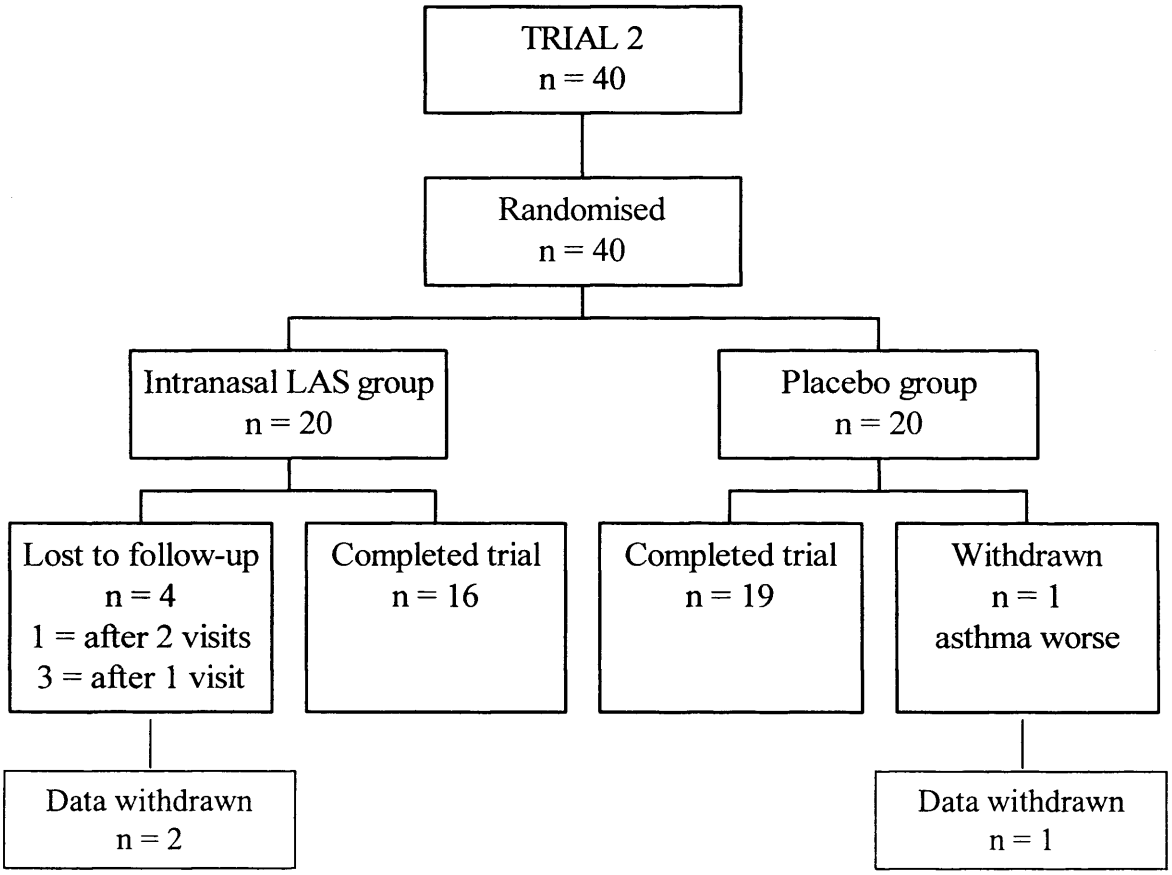


Table 5.6

Comparison of the randomised patients at baseline

Clinical feature	LAS (n = 18)	Placebo (n = 19)	p value
Age in years range (median ± S.D)	32-59 (47 ± 7.9)	28-64 (46 ± 10.7)	.71
Sex: Male (Female)	17 (1)	9 (10)	.01
Skin test positive	9	12	.41
Asthma: Yes	11	10	.66
Surgery: Yes	15	16	.96
Operations for polyps range (median ± S.D)	1-13 (2.5 ± 3.3)	1-16 (2 ± 4.2)	.76
Follow-up in months range (median ± S.D)	3-29 (12 ± 8.5)	4-33 (13.5 ± 9.6)	.27

5.4.3 *Statistical analysis*

i) Changes in laboratory measurements (Figures 5.40, 5.41, 5.42, 5.43)

Ten measures were analyzed. At each visit patients had acoustic rhinometry, rigid nasendoscopy, smell test (UPSIT), NIPF, PEFR, and a visual analogue scale for symptom score.

Changes within a group were analyzed using Wilcoxon signed-rank test, and between groups by Mann-Whitney U test. Significance values are shown in the graphs. Endoscopic nasal polyp grading was the only measure that revealed a significant change in both groups. Between group analysis of this parameter was not significant.

ii) Changes in Quality of life (QOL) measurement (Figures 5.44, 5.45)

The six domain scores were added to obtain a total. The total scores at the start and end of the trial were compared for each group. Patients receiving intranasal lysine-aspirin were significantly worse at the end of their trial period ($p = .00$, Figure 5.43). This was not the case in patients receiving placebo, and a comparison of the groups revealed a significant difference ($p = .04$).

Each domain was analyzed separately using a Mann-Whitney U test, and within group analysis was done using Wilcoxon signed-rank test. Patients receiving intranasal lysine-aspirin scored significantly worse in the following domains: 'sleep' ($p=.01$; placebo, $p=.71$; between groups, $p=.04$), and 'other symptoms' ($p=.00$; placebo, $p=.46$; between groups, $p=.01$). The difference between the groups was not significant in all other domains although patients in the lysine-aspirin group faired worse.

Figure 5.40

Changes in measurements at follow-up visits (every 3-4 months)

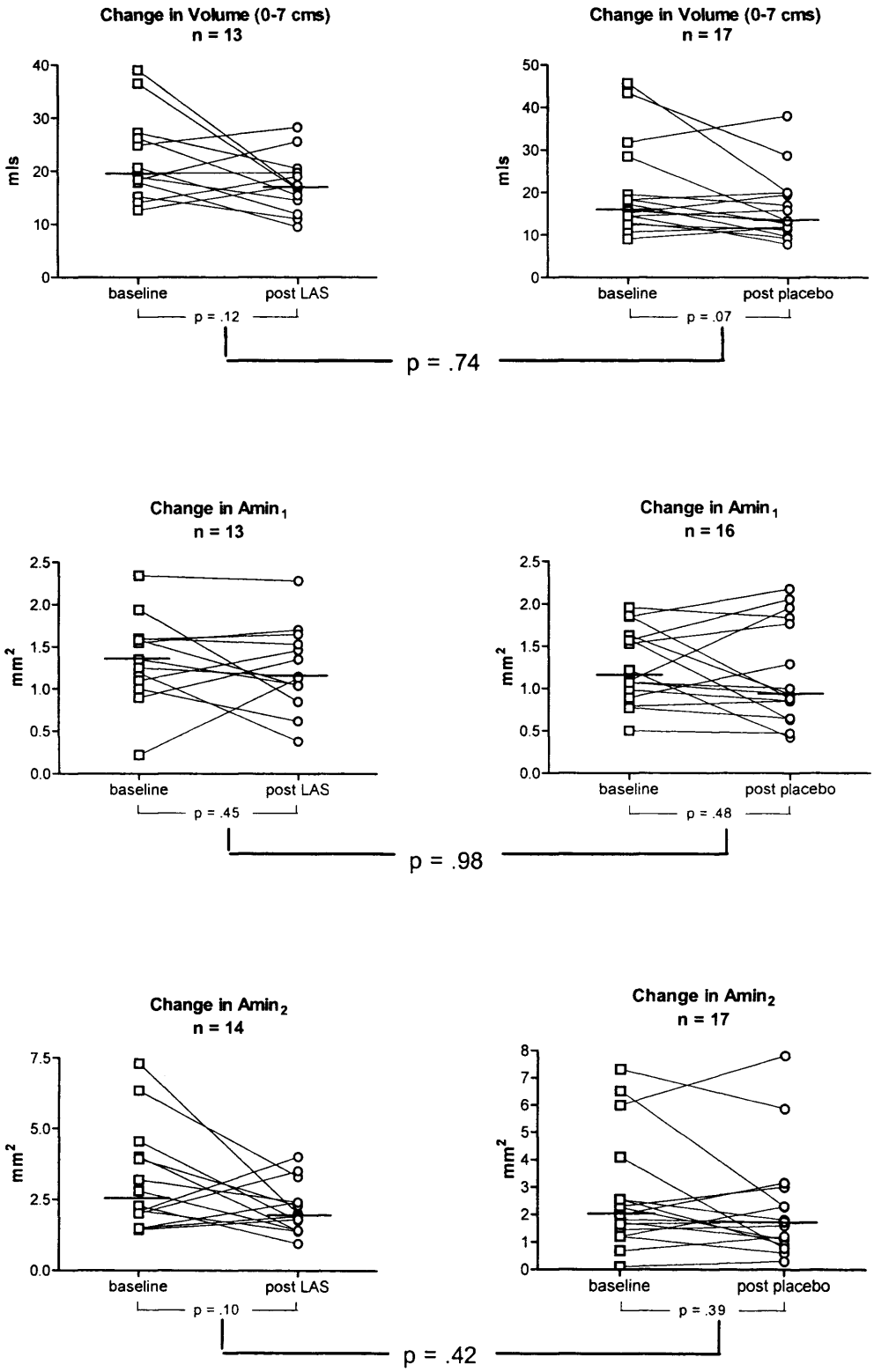


Figure 5.41

Changes in measurements at follow-up visits (every 3-4 months)

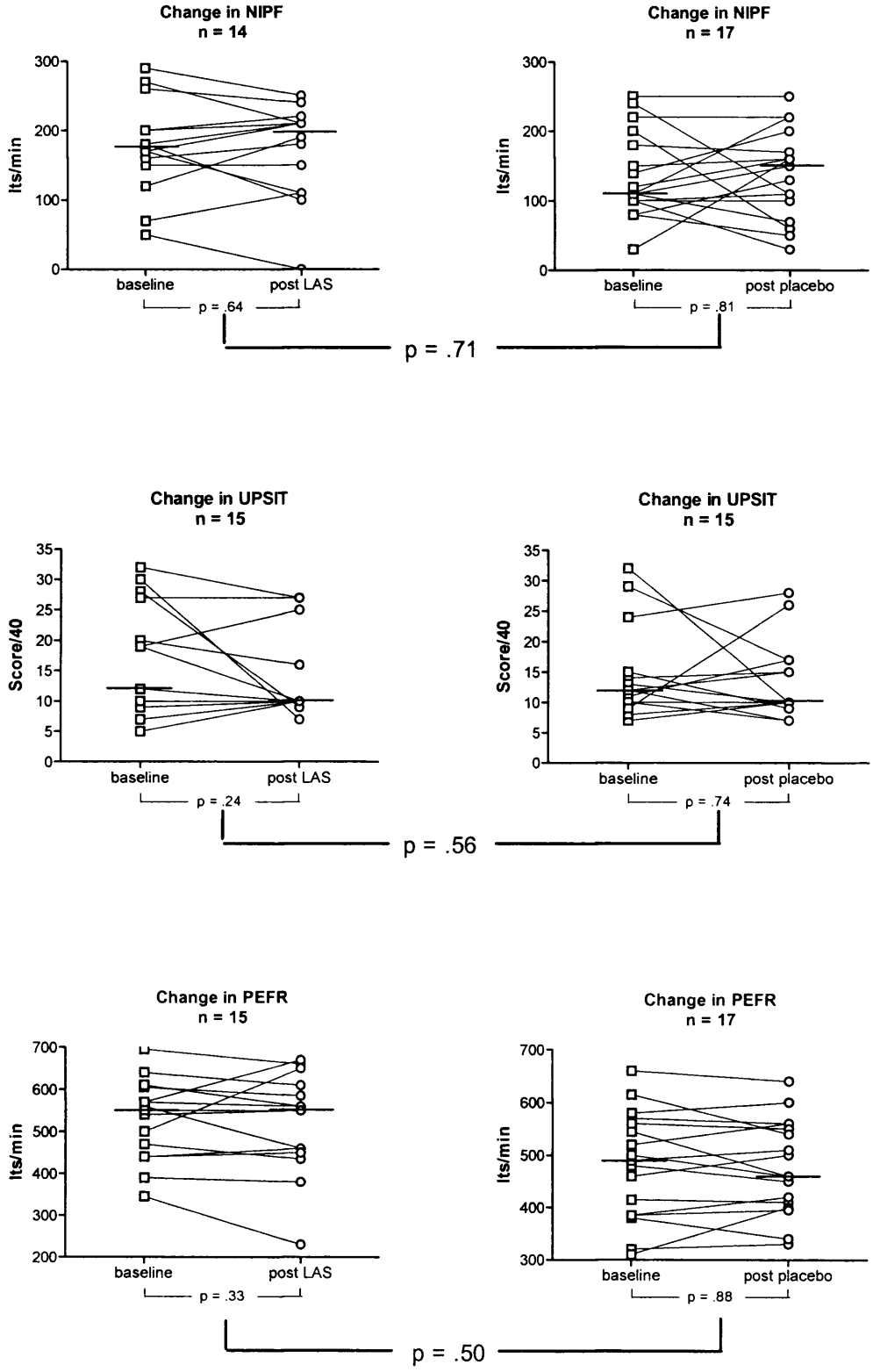


Figure 5.42

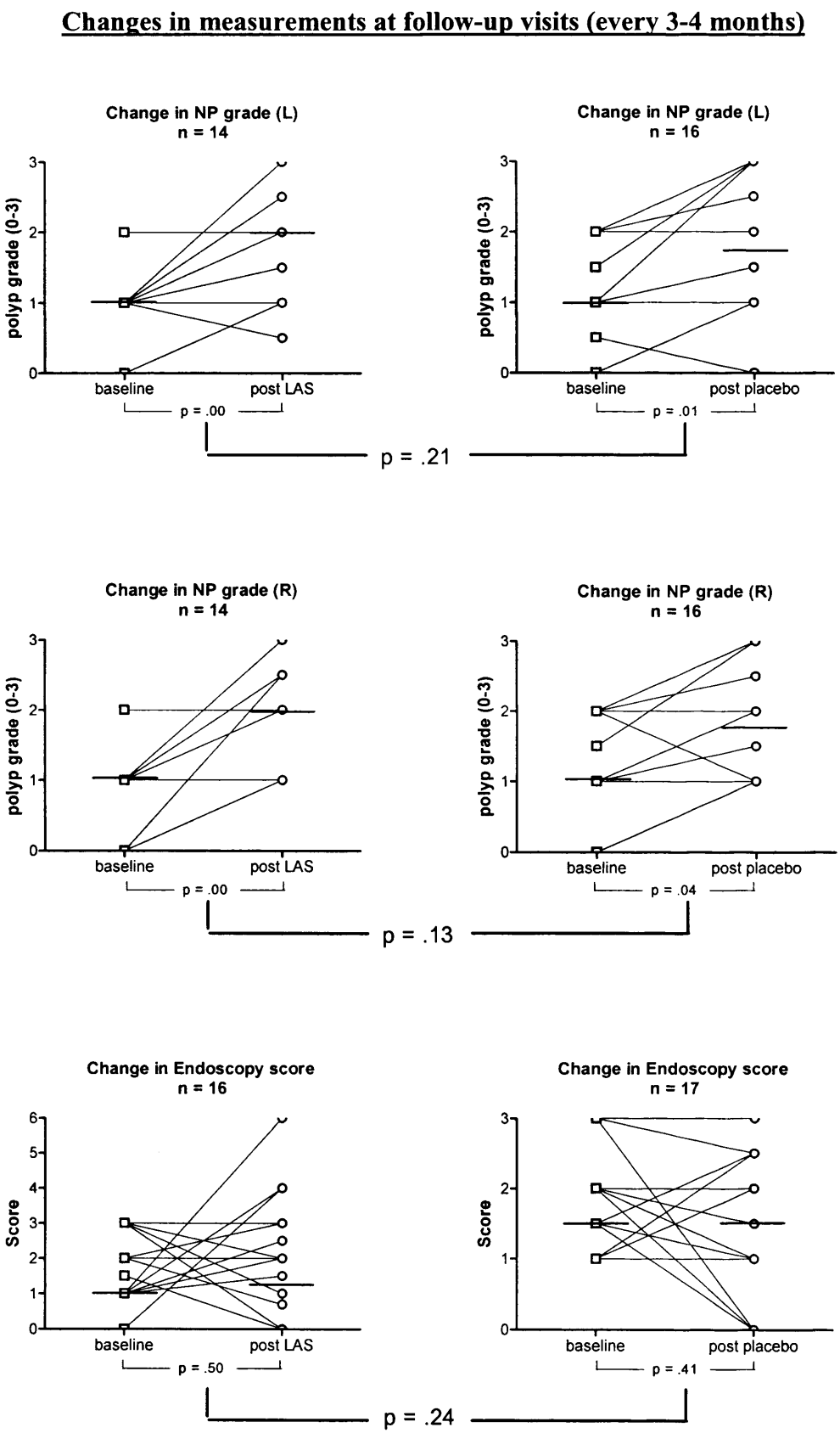

Changes in measurements at follow-up visits (every 3-4 months)
Change in NP grade (L)
n = 14
polyp grade (0-3)
baseline
post LAS
p = .00
Change in NP grade (L)
n = 16
polyp grade (0-3)
baseline
post placebo
p = .01
p = .21
Change in NP grade (R)
n = 14
polyp grade (0-3)
baseline
post LAS
p = .00
Change in NP grade (R)
n = 16
polyp grade (0-3)
baseline
post placebo
p = .04
p = .13
Change in Endoscopy score
n = 16
Score
baseline
post LAS
p = .50
Change in Endoscopy score
n = 17
Score
baseline
post placebo
p = .41
p = .24

Figure 5.43

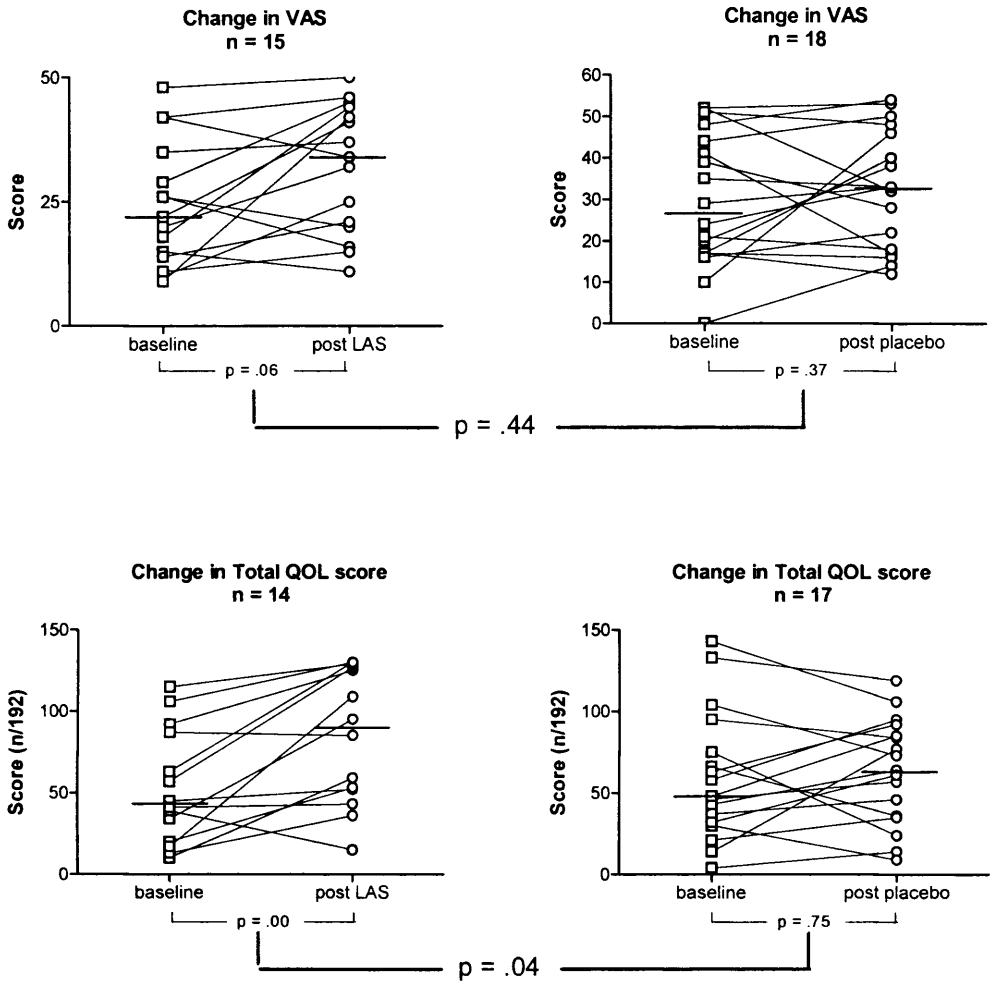

Change in VAS
n = 15
Score
50
25
0
baseline
post LAS
p = .06
Change in VAS
n = 18
Score
60
50
40
30
20
10
0
baseline
post placebo
p = .37
p = .44
Change in Total QOL score
n = 14
Score (n/192)
150
100
50
0
baseline
post LAS
p = .00
Change in Total QOL score
n = 17
Score (n/192)
150
100
50
0
baseline
post placebo
p = .75
p = .04

Figure 5.44

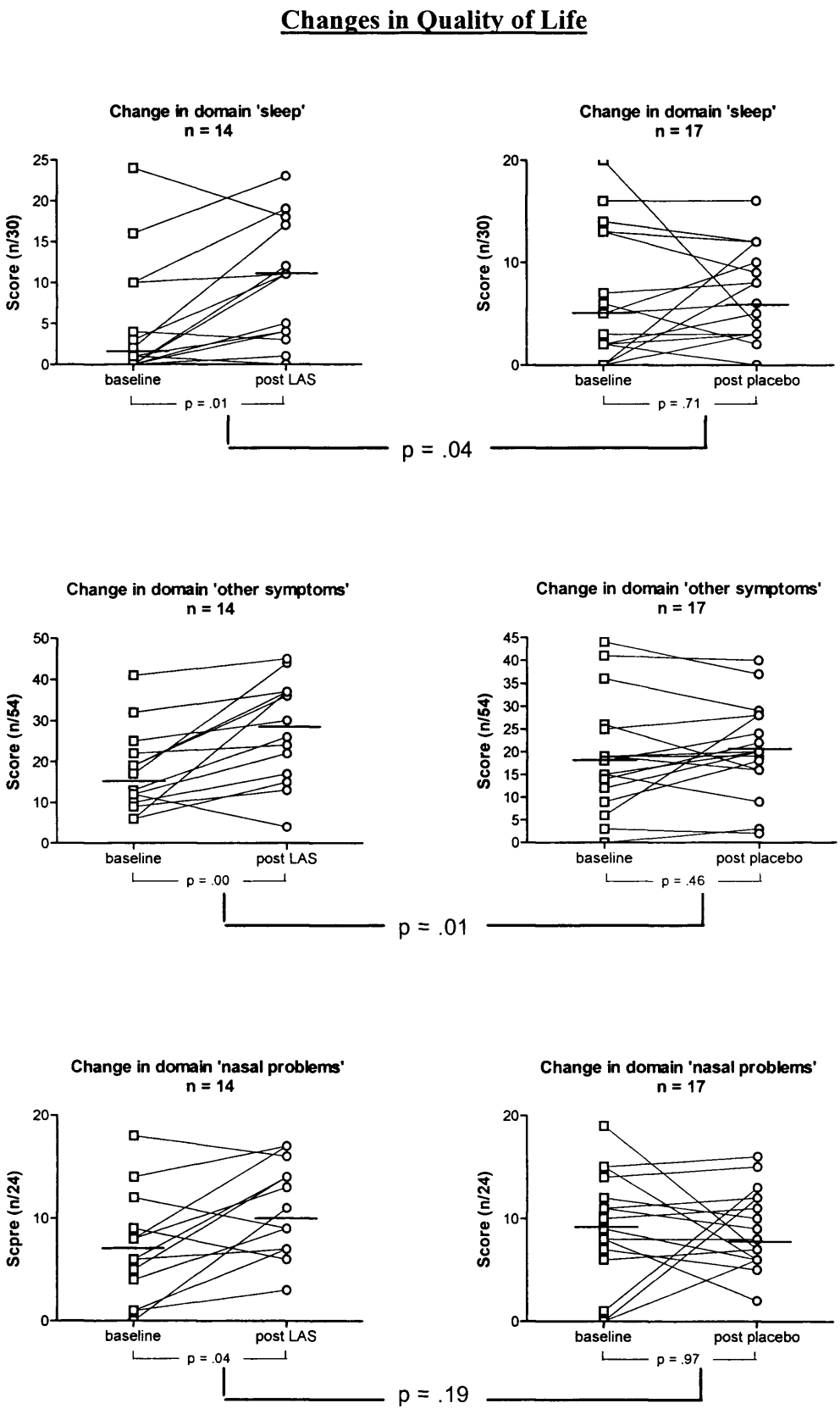
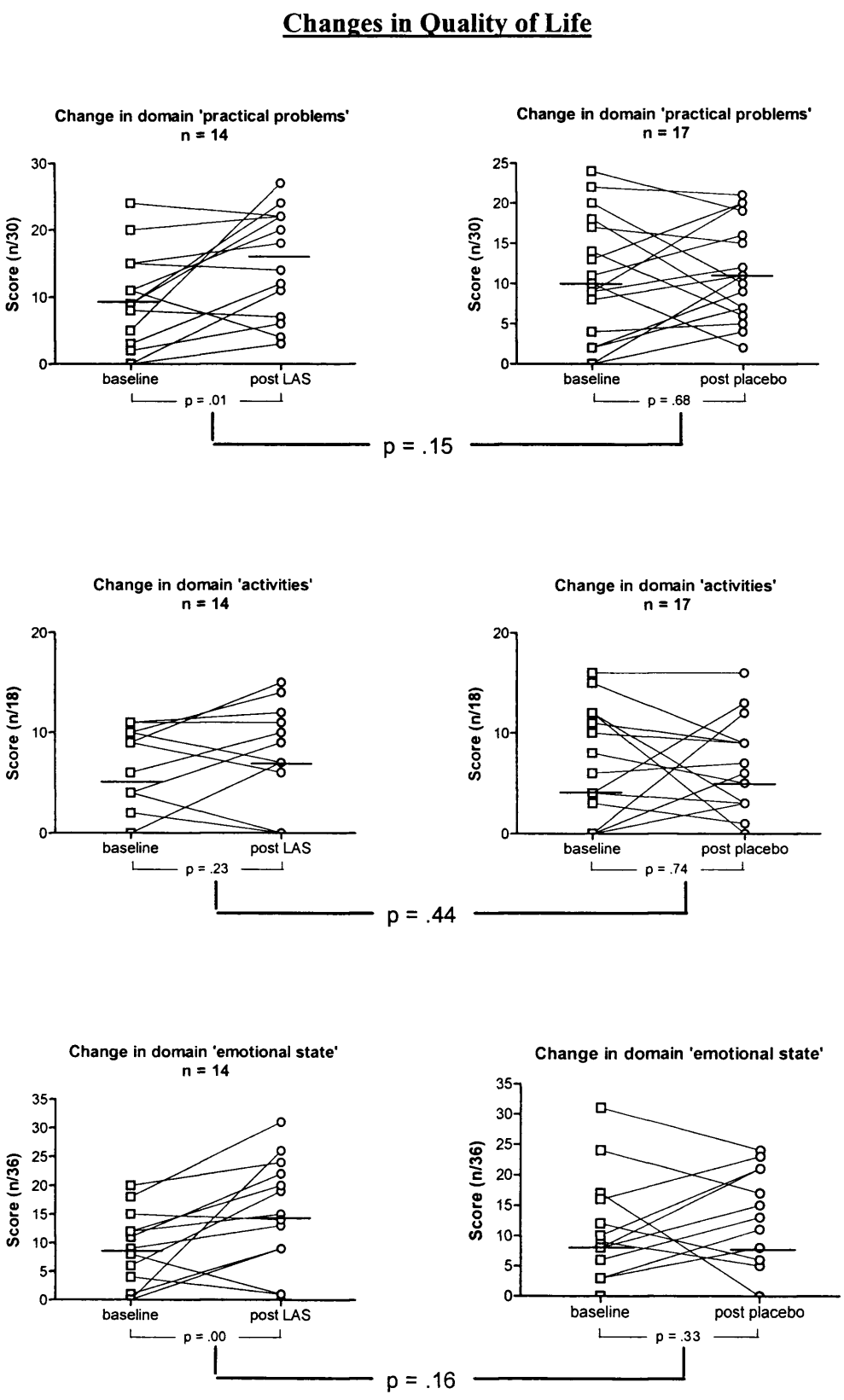

Changes in Quality of Life
Change in domain 'sleep'
n = 14
Score (n/30)
baseline
post LAS
p = .01
Change in domain 'sleep'
n = 17
Score (n/30)
baseline
post placebo
p = .71
p = .04
Change in domain 'other symptoms'
n = 14
Score (n/54)
baseline
post LAS
p = .00
Change in domain 'other symptoms'
n = 17
Score (n/54)
baseline
post placebo
p = .46
p = .01
Change in domain 'nasal problems'
n = 14
Scpre (n/24)
baseline
post LAS
p = .04
Change in domain 'nasal problems'
n = 17
Score (n/24)
baseline
post placebo
p = .97
p = .19

Figure 5.45


Changes in Quality of Life
Change in domain 'practical problems'
n = 14
Score (n/30)
baseline
post LAS
p = .01
Change in domain 'practical problems'
n = 17
Score (n/30)
baseline
post placebo
p = .68
p = .15
Change in domain 'activities'
n = 14
Score (n/18)
baseline
post LAS
p = .23
Change in domain 'activities'
n = 17
Score (n/18)
baseline
post placebo
p = .74
p = .44
Change in domain 'emotional state'
n = 14
Score (n/36)
baseline
post LAS
p = .00
Change in domain 'emotional state'
Score (n/36)
baseline
post placebo
p = .33
p = .16

5.5 Laboratory based study

5.5.1 Measurement of inducible nitric oxide synthase (iNOS) activity in polyp tissue from aspirin-sensitive, and aspirin tolerant patients.

5.5.1a *Patients*

Fifteen patients with nasal polyps undergoing routine nasal polypectomy were enrolled for this study. They were classified into 3 groups (Table 5.7). Group A comprised 7 patients without asthma or aspirin-sensitivity (3 men, age in years 46.3±9.8; mean ± S.D). Group B consisted of 3 patients with asthma who were aspirin tolerant (n=3, 1 man, age in years 40.0±6.9; mean ± S.D). Group C included 5 patients with aspirin-sensitive asthma (Samter's triad; n=5, 2 men, age in years 45.8±8.3; mean ± S.D). Aspirin-sensitive patients had undergone more polypectomies, mean 4±3, compared to 2±1 (group A), and 3±2 (group B). All asthmatics (Group B, and C) were on regular inhaled corticosteroids, and β_2-adrenoreceptor stimulants for use as required. At the time of surgery their asthma was well controlled. None of the patients were on regular oral corticosteroids.

Group C patients had a positive history of aspirin-induced reaction. They developed a respiratory type reaction (asthma ± rhinoconjunctivitis) following aspirin or NSAID ingestion. These patients had their aspirin-sensitivity confirmed by an intranasal challenge with lysine-aspirin. Patients in Groups A and B were aspirin-tolerant. Tolerance to aspirin was ascertained by direct questioning about recent aspirin/NSAID intake. This was done at their outpatient visit or by telephone.

Table 5.7

Clinical characteristics of patient groups

	Group A (n=7)	Group B (n=3)	Group C (n=5)
Nasal polyps	Yes	Yes	Yes
Asthma	No	Yes	Yes
Aspirin-sensitivity	No	No	Yes
Age in years: mean±S.D	46.3±9.8	40.0±6.9	45.8±8.3
Sex: M (F)	3 (4)	1 (2)	2 (3)
Skin test positive	4 (57%)	2 (66%)	4 (80%)
Operations for polyps: mean±S.D	2±1	3±2	4±3

S.D: standard deviation

5.5.1b *Nitric oxide synthase activity*

Nitric oxide synthase (NOS) activity was found to be present in polyp tissue from the 3 groups. There was a significantly greater amount of activity present in tissue from aspirin-sensitive individuals compared to tolerant asthmatics or non-asthmatics (Table 5.8, Figure 5.46, and Figure 5.47). Moreover the elevated level of NOS activity present in tissue from aspirin sensitive individuals was independent of calcium, indicative of the inducible form of the enzyme (iNOS). Subgroup analysis revealed a significant difference between Group C and B (Kruskal-Wallis test with Dunn's comparison of groups). Levels of NOS did not correlate with disease severity or with the occurrence of asthma.

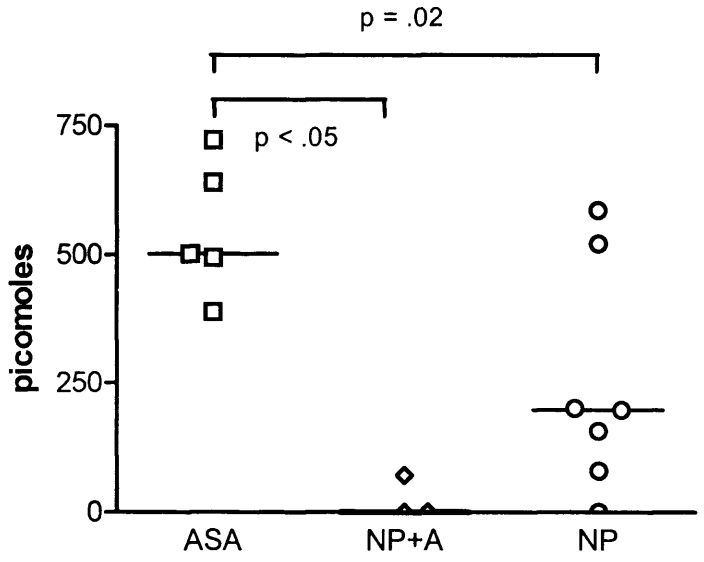
Table 5.8

Group	NOS activity median ± S.D	p value	iNOS activity median ± S.D	p value
NP + ASA (n = 5)	579.5 ± 456.3	.05	501.9 ± 32.1	.02
NP (n = 7)	96.52 ± 223.9		197.4 ± 220.8	
NP + A (n = 3)	28.97 ± 124.0		0 ± 41.06	

p value derived using non-parametric test for 3 or more groups i.e. Kruskal-Wallis. Dunn's multiple comparison test examines the relationship between the individual groups. For NOS activity none of the relationships were significant. However, for iNOS there was a significant difference between group NP+ASA and NP+A (Figure 5.46, 5.47).

Figure 5.46

iNOS activity in nasal polyp tissue



NOS activity in nasal polyp tissue

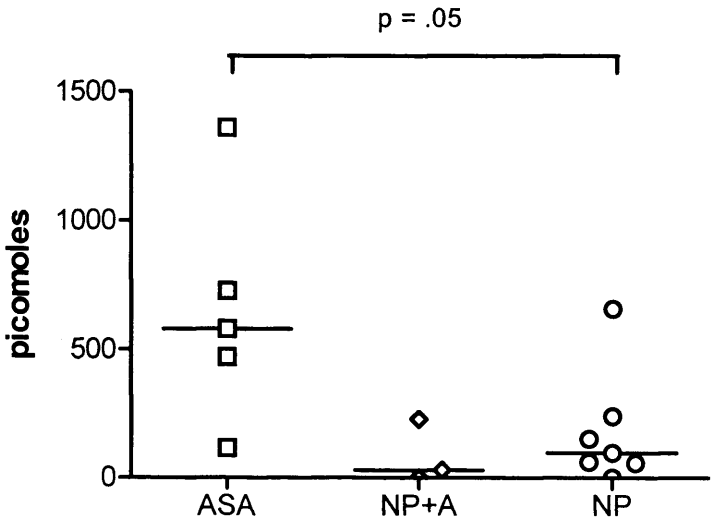
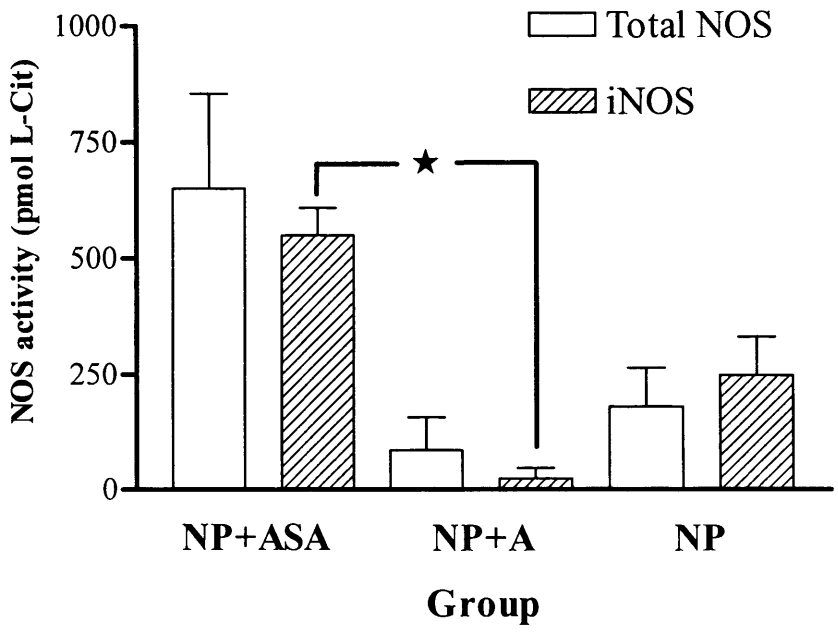


Figure 5.47

iNOS activity in nasal polyp tissue



Levels of NOS activity in homogenates of polyp tissue obtained from patients without asthma (NP), with asthma (NP+A) or with aspirin-sensitive asthma (NP+ASA). Total NOS activity (open bars) was determined in the presence of calcium. iNOS activity (hatched bars) was determined in the absence of calcium and in the presence of EGTA. iNOS activity is expressed as pmol of L-citrulline formed by samples containing equivalent levels of protein (5 mg/incubation). In each case the level of arginine converted to citrulline in the presence of 1mM L-NAME, which constituted approximately 10% of total activity, (ie non-NOS activity) has been subtracted. Data is given as the mean ± S.E. mean for n=3-7 patients. ★ represents a statistically significant difference (one way analysis of variance followed by Dunns test) in the levels of total iNOS activity in samples from aspirin sensitive patients compared to individuals with polyps and asthma.

5.5.3 Immunohistochemistry

To measure expression of the cysteinyl leukotriene$_1$ receptor and leukotriene B_4 receptor, in nasal biopsies from aspirin sensitive and aspirin tolerant subjects.
To compare the expression of cysteinyl leukotriene$_1$ receptor and leukotriene B_4 receptor in aspirin-sensitive subjects, after desensitization with topical lysine aspirin or placebo control.

5.5.3a *Aspirin-sensitive patients*

Twenty-two aspirin sensitive patients underwent nasal biopsy. Fifteen were female (7 male), with a mean age of 40.8 (S.D: 18.2; range: 20-68 years). The majority were skin prick test positive (n=15), had asthma (n=19), and had undergone surgery for nasal polyps (n=19). The mean number of operations they had undergone was 5.9 (S.D: 4.4; range: 1-20).
These 22 patients were compared to the other patients forming the aspirin sensitive group. No significant differences were noted amongst the two, which indicated that these 22 patients represented the whole aspirin sensitive group.

5.5.3b *Aspirin tolerant patients*

Twelve aspirin tolerant patients underwent nasal biopsy. Seven were male (5 female), with a mean age of 49.4 (S.D: 13.3; range: 21-69 years). Five patients were skin prick test positive, 6 had asthma, and 5 had undergone surgery for nasal polyps. The mean number of operations they had undergone was 3.4 (S.D: 2.9; range: 1-7).
These 12 patients were compared to the other patients forming the aspirin tolerant group. The only significant difference amongst the two was in the number of patients undergoing surgery, which was significantly higher in the non-biopsied group (p=. 008).

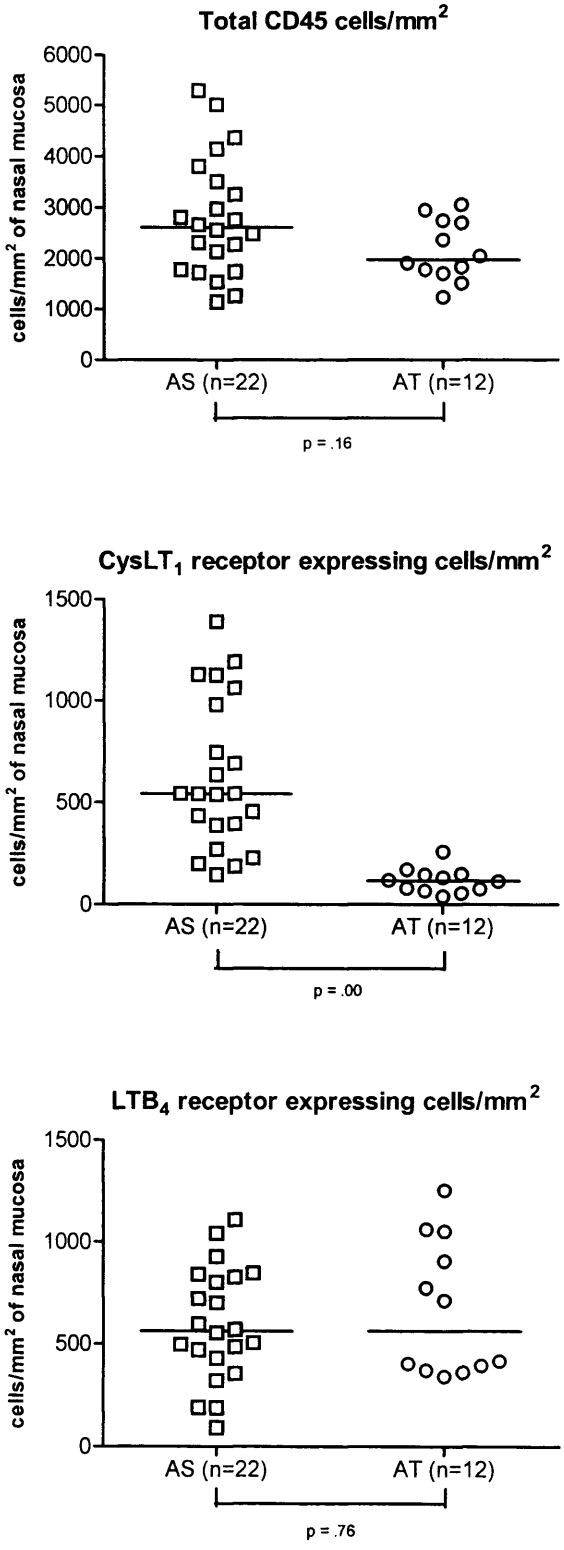
5.5.3c *CD45 count, CysLT$_1$ and LTB$_4$ receptor expression*

The total numbers of submucosal (CD45) leukocytes in aspirin sensitive and aspirin tolerant patients was not statistically different (p=.16, Figure 5.48, Table 5.9). The number of submucosal cells expressing the CysLT$_1$ receptor was significantly elevated in aspirin sensitive patients as compared to the aspirin tolerant group, although there was some overlap between the groups (p=.00, Figure 5.48, Table 5.9). In contrast, the numbers of cells expressing the LTB$_4$ receptor was not statistically different between the groups (P=.76, Figure 5.48, Table 5.9).

The percentages of the total leukocytes expressing the CysLT$_1$, but not the LTB$_4$ receptor was significantly elevated in the aspirin sensitive group as compared to the tolerant patients (p=.00, p=.23 respectively, Figure 5.49, Table 5.9).

Figure 5.48

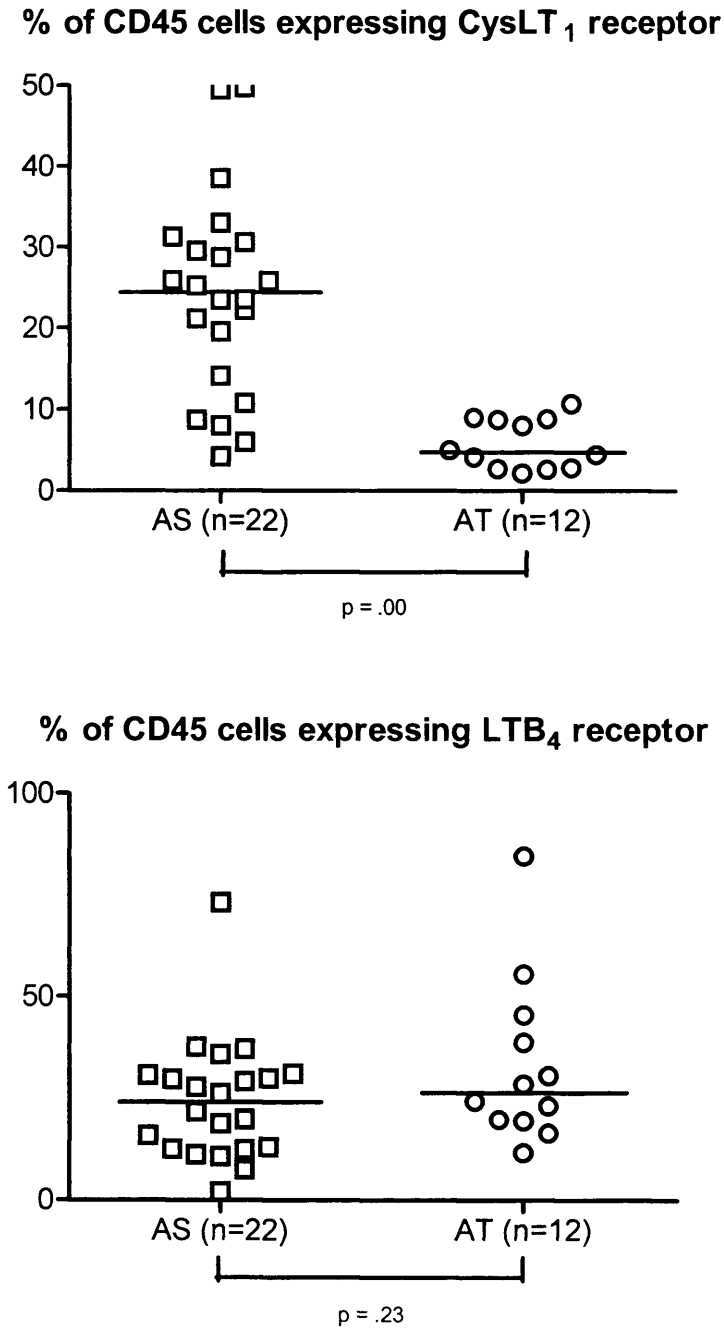
Aspirin sensitive (AS) vs. Aspirin tolerant (AT)



Horizontal lines represent medians.

Figure 5.49

Aspirin sensitive (AS) vs. Aspirin tolerant (AT)



Horizontal lines represent medians.

Table 5.9

Aspirin-sensitive vs. Aspirin tolerant (baseline data and comparison)

Cells counted	GROUP		p value
	AS (n = 22)	AT (n = 12)	
Total CD45 expressing cells/mm^2	2797.27 ± 1158.7	2154.91 ± 595.03	.16
Total $CysLT_1$ receptor expressing cells/mm^2	628.82 ± 367.37	117.92 ± 60.77	.00
Total LTB_4 receptor expressing cells/mm^2	594.32 ± 277.1	669.75 ± 331.45	.76
% of CD45 cells expressing $CysLT_1$ receptor	24.09 ± 12.48	5.79 ± 3.07	.00
% of CD45 cells expressing LTB_4 receptor	24.43 ± 14.96	33.31 ± 20.5	.23

5.5.3d *Phenotypes of cells expressing $CysLT_1$ and LTB_4 receptor*

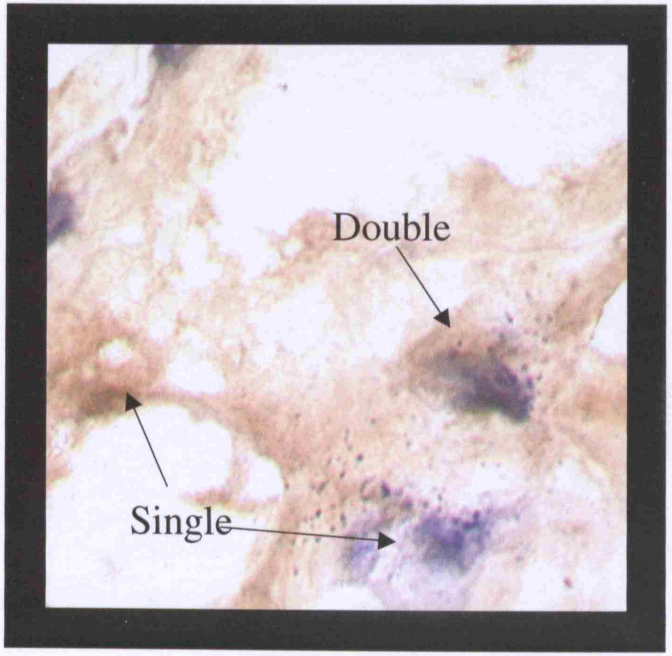
Macrophages, T cells, eosinophils, neutrophils and mast cells respectively accounted for a similar proportion of the total nasal submucosal cells expressing the $CysLT_1$ receptor, and the LTB_4 receptor in aspirin sensitive and aspirin tolerant patients (Figure 5.50, Table 5.10).

Table 5.10

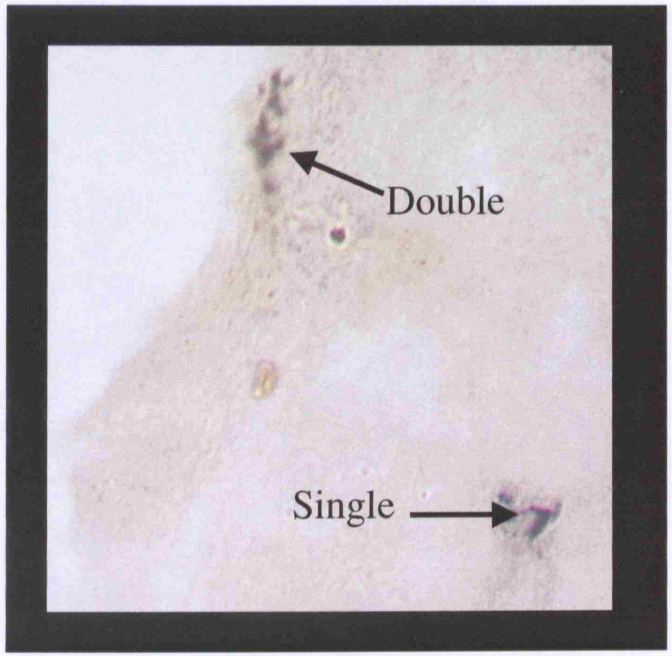
Percentages of total LTC_1 and LTB_4 receptor-positive cells co-expressing leukocyte phenotypic markers in the nasal mucosa of 3 AS and 3 AT patients. Data are expressed as the mean (range)

Leukocyte	% Of total LTB_4 receptor expressing cells		% Of total LTC_1 receptor expressing cells	
	AS	AT	AS	AT
Macrophages	29.1 (24.8 - 32.2)	29.8 (28.0 - 30.8)	31.6 (28.1 - 35.1)	30.3 (23.6 - 36.2)
T cells	32.1 (31.8 - 32.8)	31.6 (29.8 - 32.7)	25.2 (18.5 - 33.1)	20.1 (18.6 - 22.8)
Eosinophils	29.6 (24.5 - 32.8)	26.6 (23.6 - 32.3)	20.9 (18.1 - 22.7)	20.5 (16.8 - 24.2)
Neutrophils	0.6 (0.5 - 0.6)	1.6 (1.3 - 1.9)	19.5 (10.2 - 32.2)	20.6 (13.2 - 25.5)
Mast cells	7.2 (5.2 - 9.2)	7.9 (4.3 - 11.5)	0.6 (0.4 - 0.7)	0.7 (0.5 - 1.0)
Total	98.6	97.5	97.8	92.2

Figure 5.50



Double staining for LTB_4 receptor (brown) and Neutrophils (blue)



Double staining for $CysLT_1$ receptor (brown) and Neutrophils (blue)

5.5.3e *Effect of desensitization with lysine-aspirin or placebo on numbers of cells expressing the $CysLT_1$ and LTB_4 receptors*

i. 6 month desensitization

During the trial of desensitization (Trial 1; section 5.3), nasal biopsies were obtained from consenting patients at baseline and at the end of their 6-month phase. Three patients agreed to a biopsy at each stage, 2 at the end of the intranasal lysine-aspirin phase, and 1 at the end of the placebo phase. Thus, we had 5 pairs to compare baseline and post lysine-aspirin and 4 pairs to compare baseline and post placebo.

Table 5.11 and figure 5.51, 5.52 show the changes in the different cell counts after active or placebo treatment. There was tendency towards reduction in the total number of cells expressing $CysLT_1$ receptors (p=.13), which was more obvious when we narrowed our focus to CD45 leukocytes expressing $CysLT_1$ receptors (p=.06). Following placebo no significant changes were seen.

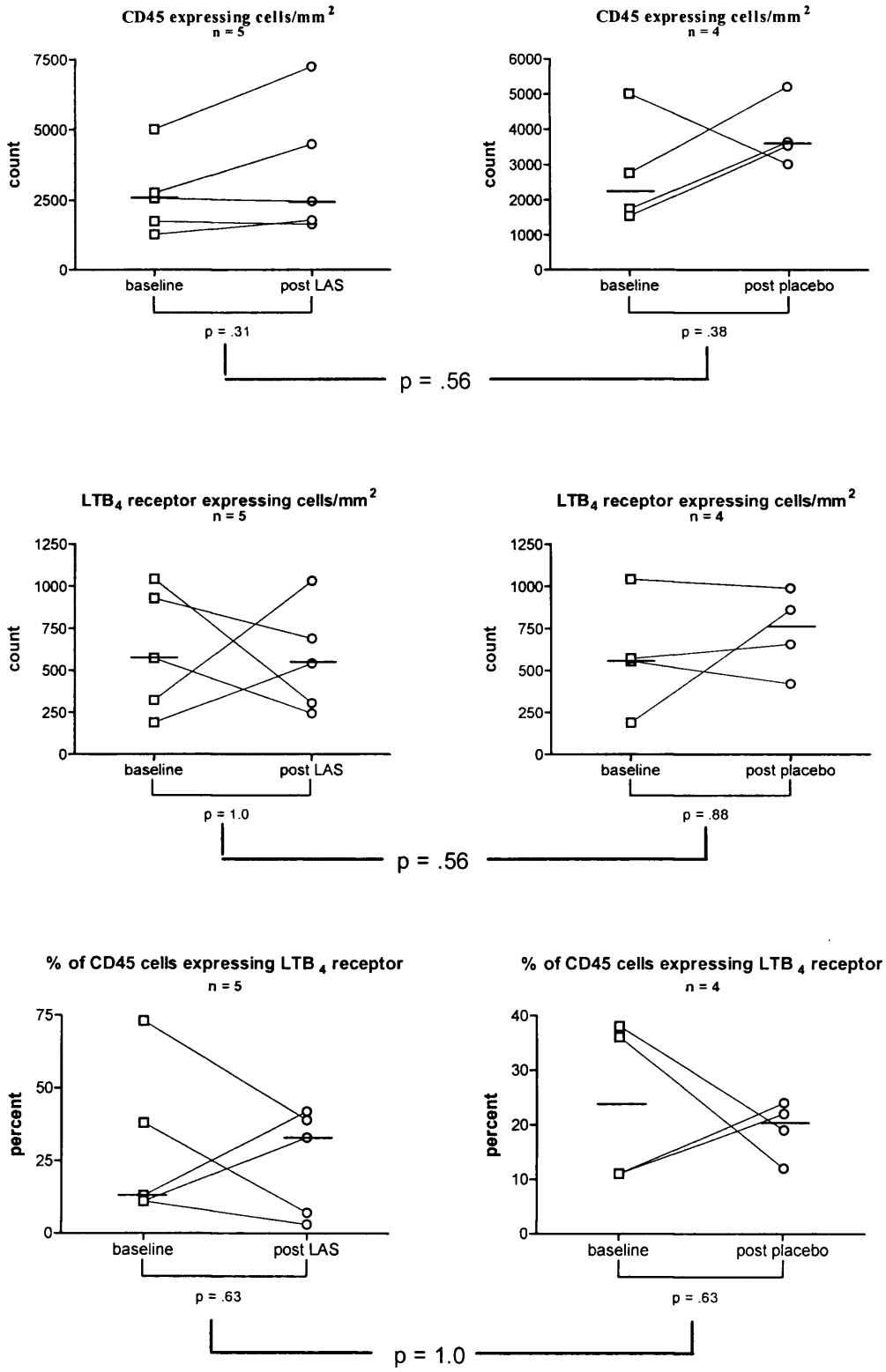
The reduction in $CysLT_1$ receptors both total, and as a percentage of CD45 was significantly greater in the intranasal lysine-aspirin phase when compared to the placebo phase (p=.03, and p=.03 respectively; Figure 5.52).

Table 5.11: Effect of long term desensitization (6 months)

Count		Baseline	Post LAS	p
Total CD45 expressing cells/mm^2	n = 5	2477.45 ± 1368.33	3513.72 ± 2369.96	.31
Total $CysLT_1$ receptor expressing cells/mm^2		588.50 ± 240.66	252.00 ± 281.74	.13
Total LTB_4 receptor expressing cells/mm^2		600.67 ± 332.71	562.00 ± 318.31	1.0
% of CD45 cells expressing $CysLT_1$ receptor		.27 ± .09	.07 ± .04	.06
% of CD45 cells expressing LTB_4 receptor		.30 ± .24	.25 ± .18	.63
		Baseline	**Post placebo**	**p**
Total CD45 expressing cells/mm^2	n = 4	2477.45 ± 1368.33	3847.37 ± 946.17	.38
Total $CysLT_1$ receptor expressing cells/mm^2		588.50 ± 240.66	1820.25 ± 1967.85	.25
Total LTB_4 receptor expressing cells/mm^2		600.67 ± 332.71	732.25 ± 248.69	.88
% of CD45 cells expressing $CysLT_1$ receptor		.27 ± .09	.41 ± .34	.63
% of CD45 cells expressing LTB_4 receptor		.30 ± .24	.19 ± .05	.63
		% change post LAS	**% change post placebo**	**p**
Total CD45 expressing cells/mm^2	n = 5 vs. 4	27.35 ± 30.74	71.98 ± 76.38	.56
Total $CysLT_1$ receptor expressing cells/mm^2		-54.16 ± 52.95	135.63 ± 150.23	**.03**
Total LTB_4 receptor expressing cells/mm^2		51.07 ± 141.64	86.01 ± 182.01	.56
% of CD45 cells expressing $CysLT_1$ receptor		-63.56 ± 36.55	42.72 ± 68.94	**.03**
% of CD45 cells expressing LTB_4 receptor		44.44 ± 153.29	25.38 ± 97.19	1.0

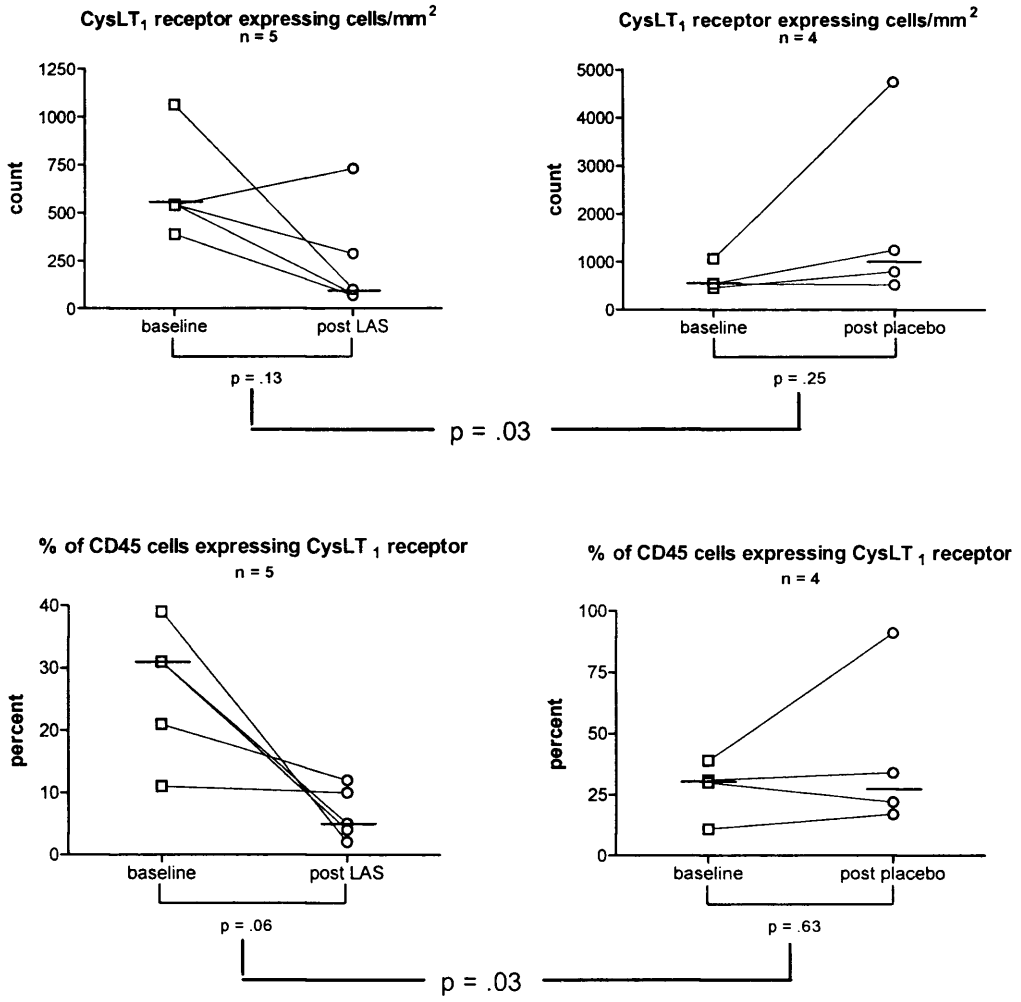
Figure 5.51

Effect of long term desensitization (6 months)



Horizontal lines represent medians.

Figure 5.52



Horizontal lines represent medians.

ii. 2 week desensitization

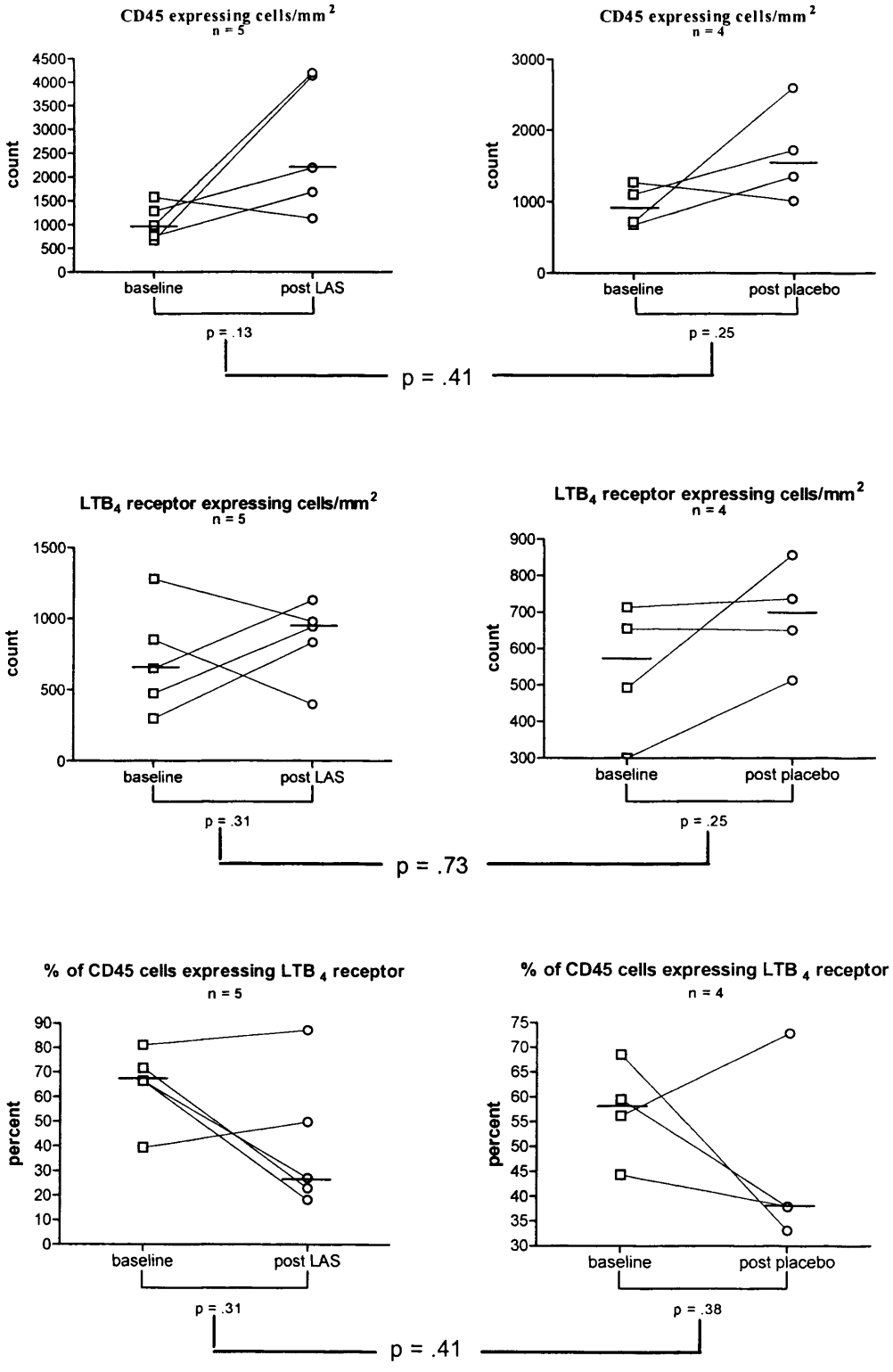
Nine patients agreed to have nasal biopsy following a 2-week course of either intranasal lysine-aspirin (n=5) or placebo (n=4) in order to study the effects of short-term desensitization. Table 5.12, figures 5.53, and 5.54 show the changes in the different cell counts after active or placebo treatment. CD45 leukocytes expressing There was a tendency towards significant reduction in $CysLT_1$ receptors following intranasal lysine-aspirin (p=.06). Following placebo no significant changes were seen.

Table 5.12: Effect of short term desensitization (2 weeks)

Count		Baseline	Post LAS	p
Total CD45 expressing cells/mm^2	n = 5	1051.02 ± 379.51	2660.83 ± 417.96	.13
Total $CysLT_1$ receptor expressing cells/mm^2		455.64 ± 248.86	608.52 ± 430.23	.63
Total LTB_4 receptor expressing cells/mm^2		709.95 ± 378.59	856.23 ± 278.21	.31
% of CD45 cells expressing $CysLT_1$ receptor		41.72 ± 9.96	21.33 ± 5.38	.06
% of CD45 cells expressing LTB_4 receptor		64.95 ± 15.53	40.93 ± 28.47	.31
		Baseline	**Post placebo**	**p**
Total CD45 expressing cells/mm^2	n = 4	942.85 ± 290.34	1669.05 ± 680.29	.25
Total $CysLT_1$ receptor expressing cells/mm^2		535.29 ± 250.89	675.06 ± 477.59	.88
Total LTB_4 receptor expressing cells/mm^2		540.42 ± 185.01	689.11± 145.04	.25
% of CD45 cells expressing $CysLT_1$ receptor		55.49 ± 18.09	41.50 ± 26.25	.37
% of CD45 cells expressing LTB_4 receptor		57.09 ± 10.00	45.39 ± 18.40	.38
		% change post LAS	**% change post placebo**	**p**
Total CD45 expressing cells/mm^2	n = 5 vs. 4	203.37 ± 222.14	98.85 ± 118.74	.41
Total $CysLT_1$ receptor expressing cells/mm^2		90.24 ± 178.10	51.23 ± 101.44	.91
Total LTB_4 receptor expressing cells/mm^2		55.35 ± 94.82	36.72 ± 40.99	.73
% of CD45 cells expressing $CysLT_1$ receptor		-46.64 ± 16.69	-24.51 ± 38.26	.91
% of CD45 cells expressing LTB_4 receptor		-33.29 ± 46.49	-18.22 ± 35.40	.41

Figure 5.53

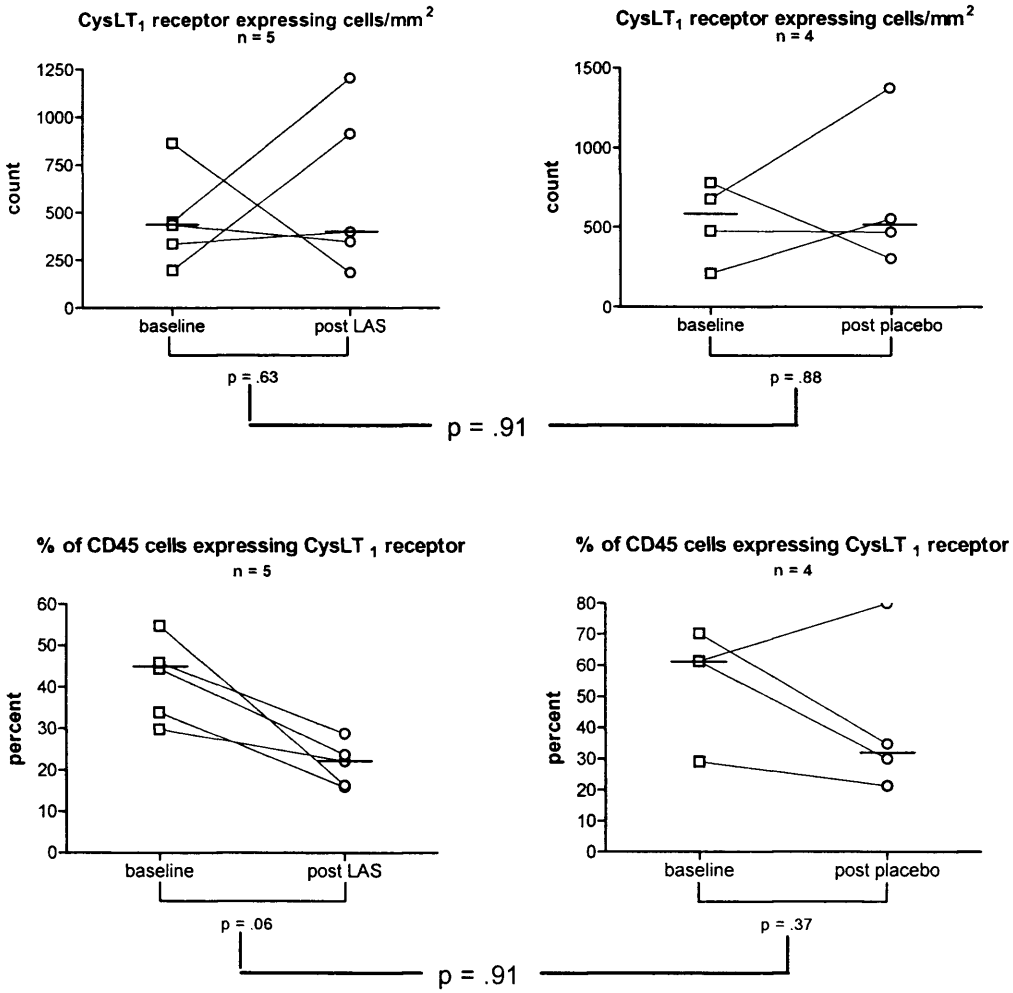
Effect of short term desensitization (2 weeks)



Horizontal lines represent medians.

Figure 5.54

Effect of short term desensitization (2 weeks)



Horizontal lines represent medians.

iii. Combined data

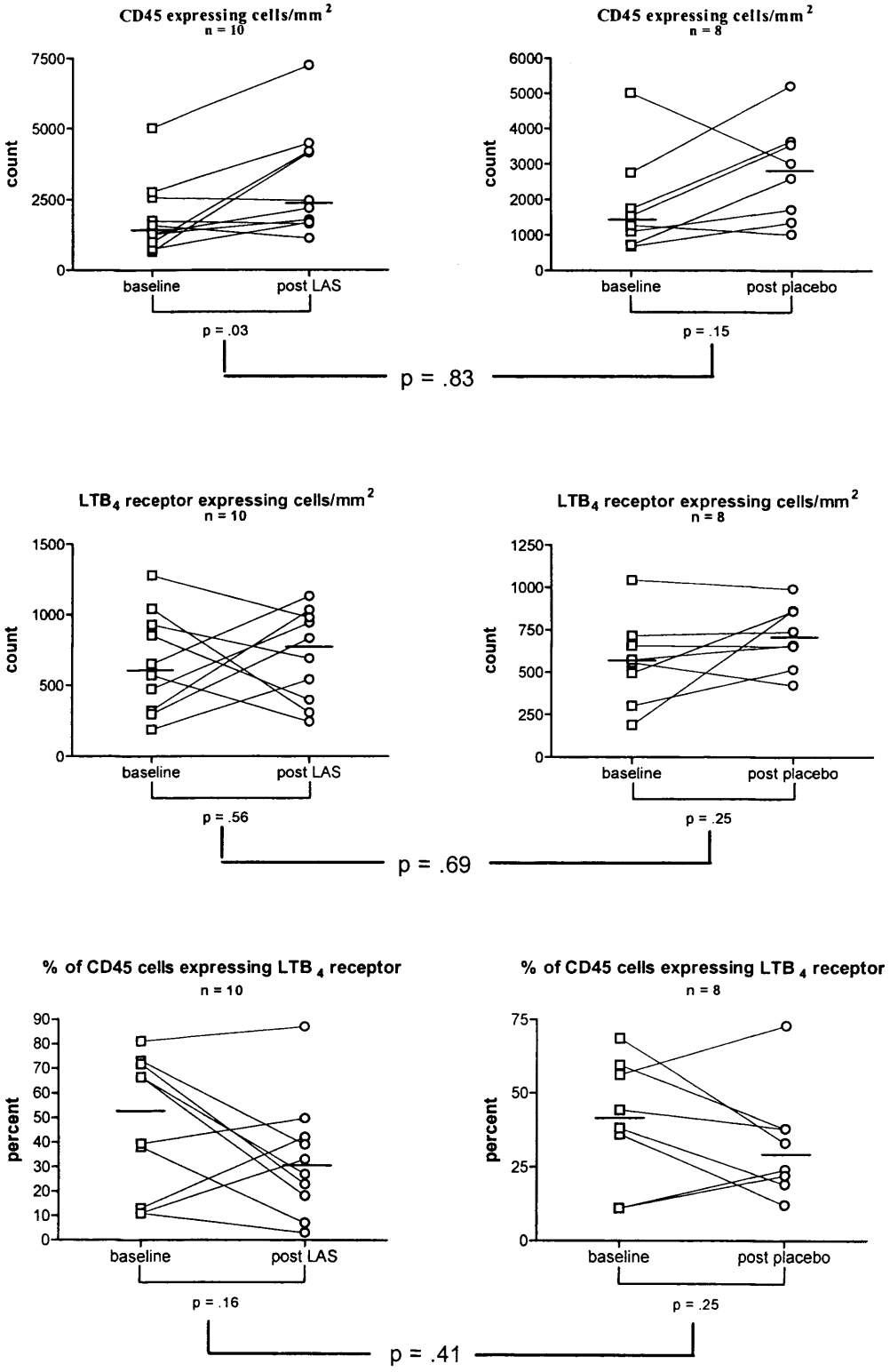
Since the trends in these changes following either 2 weeks or 6 months of treatment with lysine-aspirin or placebo were identical, the 2 groups were amalgamated for the purpose of further statistical analysis.

Identical trends were confirmed with no significant differences observed between the 2-week and 6-month treatment groups. Groups were compared for changes in absolute CD45 leukocyte counts, absolute numbers of $CysLT_1$ receptor expressing, LTB_4 receptor expressing cells, percentages of CD45 leukocytes expressing $CysLT_1$ receptor, and percentages of CD45 leukocytes expressing LTB_4 receptor.

The combined data are shown in Figure 5.55, 5.56 and representative sections are shown in Figure 5.57. In the patients treated with lysine-aspirin there was a small but significant (p=.03) increase in the total CD45 leukocytes in the nasal mucosa which was not seen in the patients treated with placebo. However, the difference between the changes in each group were not significant (p=.83). Treatment with lysine-aspirin was associated with a reduction in the percentages of CD45 leukocytes expressing $CysLT_1$ receptor in all 10 patients so treated (p=.002). The response to placebo treatment was heterogeneous but showed no overall significant change. The differences between the changes in the percentages of CD45 leukocytes $CysLT_1$ receptor in the lysine-aspirin and placebo-treated groups were also significant (p=.02). There were no significant changes in the percentages of CD45 leukocytes expressing the LTB_4 receptor in association with lysine-aspirin or placebo therapy, and the differences in the changes seen in each group were likewise not significant.

Figure 5.55

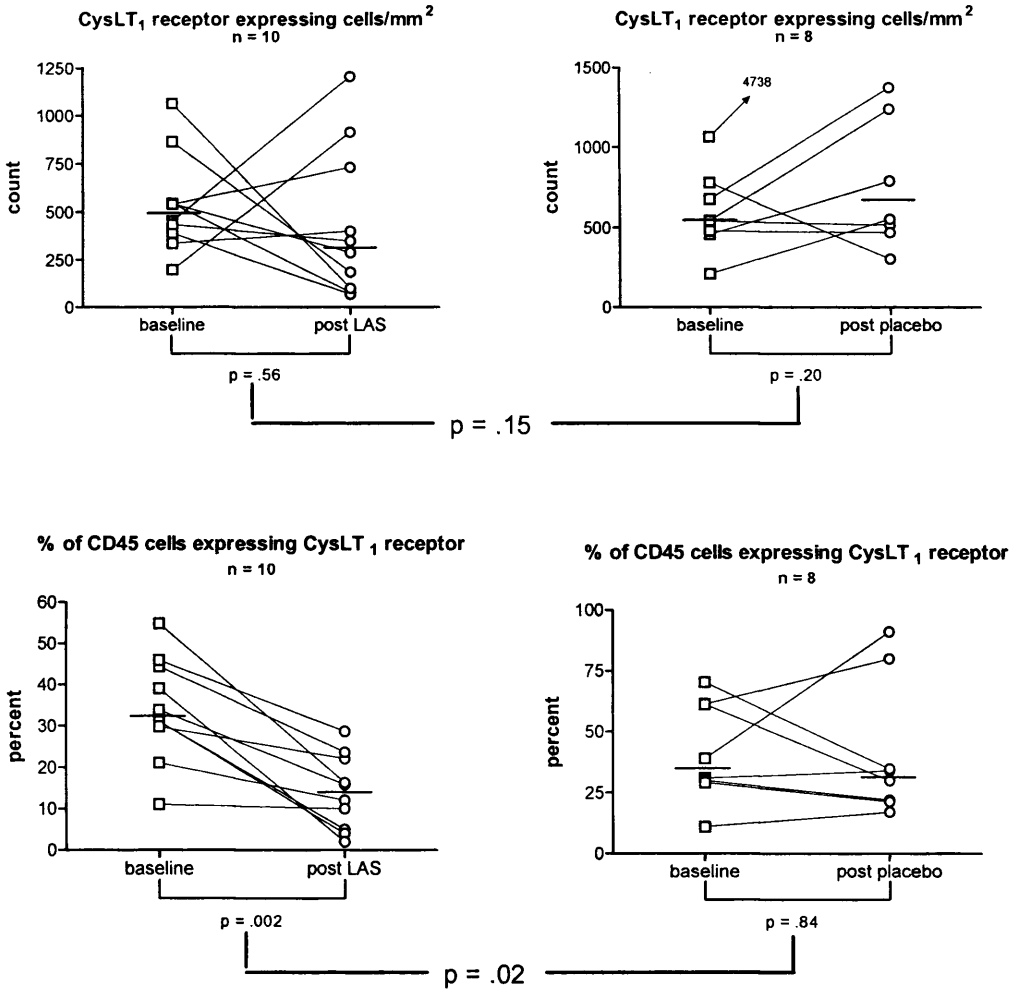
Effect of desensitization (long term and short term)



Horizontal lines represent medians.

Figure 5.56

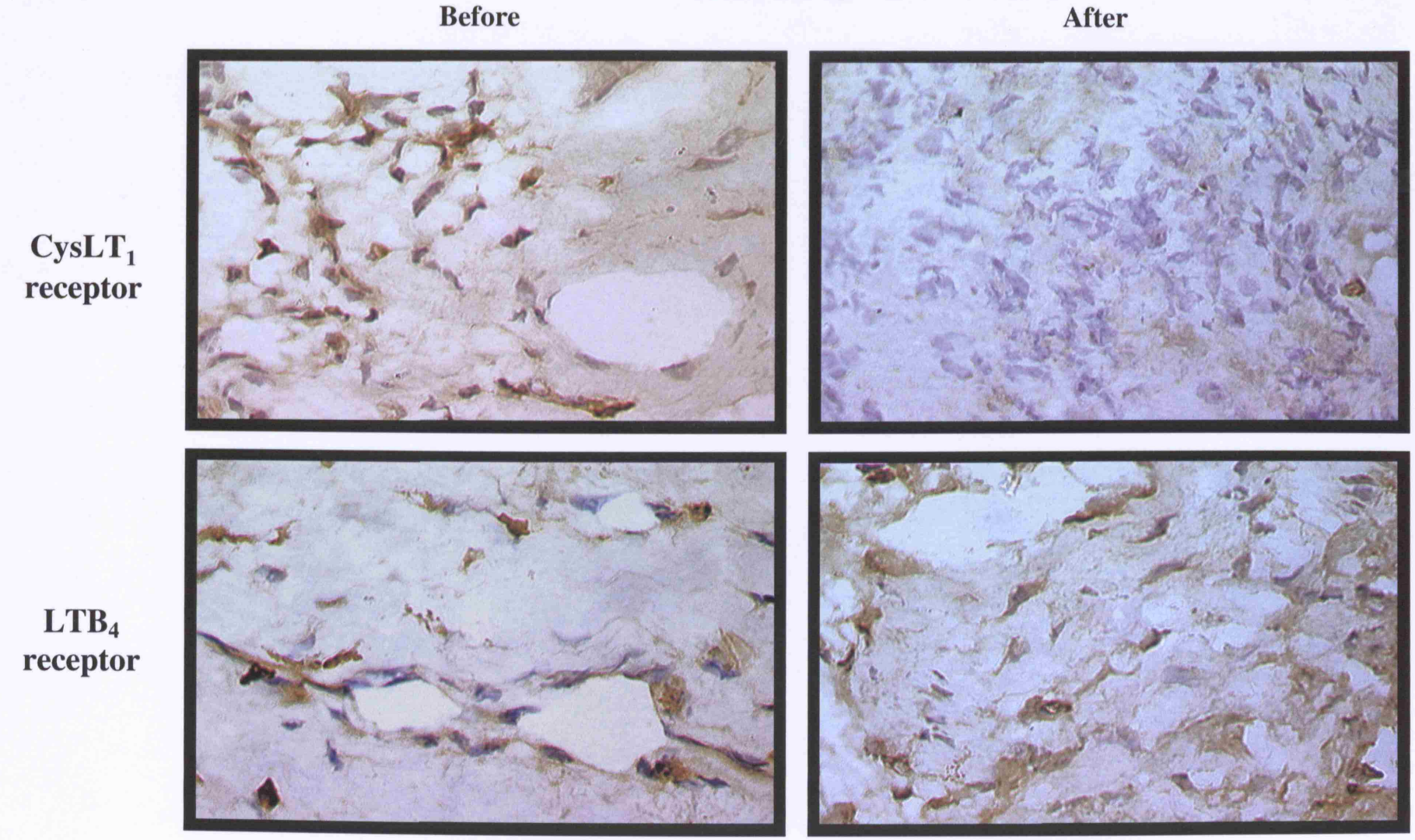
Effect of desensitization (long term and short term)



Horizontal lines represent medians.

Figure 5.57

DESENSITISATION



DISCUSSION

6.1 *Intranasal LAS in AS patients with nasal polyposis: clinical trial*

6.1.1 Primary Aim

We studied the effects of alternate day, low dose (16 mgs) intranasal lysine-aspirin in patients with aspirin-sensitive nasal polyps. The clinical effectiveness of this therapeutic option in controlling nasal symptoms and polyp growth rate were evaluated by conducting a randomised, double blind, placebo-controlled, crossover trial. We also collected nasal turbinate tissue prior to and during the trial to examine the changes that may be occurring at a molecular level.

Immunohistochemical analysis has shown for the first time that in patients with aspirin-sensitive nasal polyposis there is a significant selective increased expression of $CysLT_1$ receptor on nasal submucosal inflammatory cells, and that low dose intranasal lysine-aspirin leads to a significant reduction in the number of cells expressing this receptor when compared to placebo.

However, analysis of the clinical parameters failed to demonstrate any significant benefit on nasal symptoms or polyp growth rate.

6.1.2 Rationale for the study

Since the initial report on tolerance to aspirin after a positive oral challenge (Stevenson et al. 1980), and an improvement in rhinitis and asthma following its continued use, several studies have been published to evaluate the clinical effectiveness of regular aspirin (Table 6.1). Majority of these studies concluded that regular intake of aspirin improved the patients nasal symptoms and controlled polyp growth rate. Gastrointestinal side effects were reported by many studies with percentages ranging from 8-46%. To avoid these side effects a new route, intranasal, was employed for desensitization (Patriarca et al. 1991b). This study demonstrated the beneficial effects of low dose intranasal lysine-aspirin on nasal symptoms and polyp growth.

Table 6.1

Trials of desensitization

Group	Year	Type of study	Method	Dose (mgs)	n=	Period (mths)	Outcome measures	Results
Stevenson	1980	Observation	Oral	325-650	2	8-9	FEV_1	Stable
							Oral CS use	Reduced
							Nasal CS use	Same
							Nasal symptoms (arbitrary scale 0-4)	Improved
Chiu	1983	Prospective	Oral	173-807	12	4	Subjective asthma status	Improved = 50%
								No change = 25%
								Worse = 25%
							Oral CS use	Stopped = 1 (8%)
							Inhaled CS use	Stopped = 3 (24%)
Lumry	1983	Prospective	Oral	325-2600	17	12	Patient reported improvement:	Improved =11 (47%)
							In nasal symptoms & asthma	Initial improvement = 2 (12%)
								No improvement = 4 (23%)
Stevenson	1984	R, DB, PC, CO	Oral	325-2600	25	7	Daily nasal symptoms (arbitrary scale 1-5)	Improvement = 67%
							Daily chest symptoms (arbitrary scale 1-5)	Improvement = 48%
							FEV_1 monthly	No comment
							Oral CS use	Reduced = 36%, Same = 20%,
								More = 24%
Naeije	1984	Prospective	Oral		10	10	FEV_1	No improvement
							Oral/Inhaled CS use	No improvement
Kowalski	1986	Prospective	Oral	600	14	1	Nasal symptom score (no scale)	Improvement = 8 (57%)
							Asthma score (no scale)	Improved = 7 (50%)
							Response to inhaled histamine	Improved = 10 (71%)

Group	Year	Type of study	Method	Dose (mgs)	n=	Period (mths)	Outcome measures	Results
Sweet	1990	Retrospective, with controls	Oral	325-2600	107	25-102	Hospital visits	Reduced (p=. 01)
							Oral/inhaled/nasal CS use	Reduced (p<. 01)
							Smell sense	Improved (p<. 01)
							Nasal symptoms (arbitrary scale 1-5)	Reduced (p<. 01)
							Chest symptoms (arbitrary scale 1-5)	Reduced (p=. 10)
							Number of polypectomies	Less (p=. 04)
Patriaca	1991	Prospective, with controls	Intranasal	2	28	24	Relapse of polyps	Reduced (p<. 0001)
Schapowal	1994	R, DB, PC	Intranasal	100	12	1	Nasal symptoms (arbitrary scale 0-4)	No significant difference on all counts when compared to placebo group
							Nasal polyps	
							Smell sense	
Stevenson	1996	Prospective	Oral	325-1950	29	12-36	Attacks of sinusitis/year	Less (p=. 0001)
					36	36-72	Sinus surgery/year	Less (p=. 001)
							Hospital visits	Less (p=. 005)
							Oral/inhaled/nasal CS use	Reduced (p=. 002)
							Smell sense	Improved (p<. 0001)
Nucera	2000	Prospective, with controls	Intranasal	4	38	72	Recurrence of nasal polyps	Less (p<. 001)
Gosepath	2001	Prospective	Oral	100	30	12	Recurrence of nasal polyps	Reduced
							Attacks of sinusitis	Reduced
							Lung function	Improved
							Nasal blockage, Smell sense	Improved

We decided to undertake a similar trial at our hospital where the specialist rhinology clinics (led by my internal supervisor Dr. Glenis Scadding) managed a large population of patients with nasal polyps many of whom were aspirin-sensitive. These patients were treated with traditional methods that included medical polypectomy (a course of oral corticosteroids), long-term intranasal corticosteroids, and surgical polypectomy when required. An alternative therapeutic option was very welcome and to explore the possibilities in detail we designed this trial.

6.1.3 Design of our study

Desensitization using aspirin intranasally has been attempted only on one occasion (Patriarca et al. 1991b). Despite the success there are several criticisms of the trial. The patients were not randomised or blinded. Their publication does not mention the criteria for allocating patients to the active treatment or control group, and if there was any attempt to match them at baseline. Also, the use of adjuvant intranasal medication by the groups during their trial period is unclear.

To address these issues we designed a randomised, double blind, placebo-controlled trial. During our trial patients were requested to stop all other intranasal medication. We were available to see our patients between scheduled visits if they deteriorated. Our clinical and laboratory assessment helped us decide if withdrawal was warranted.

Recruiting an adequate number of patients for a long-term study can be difficult (Stevenson et al. 1996). Allocating the patients into the active treatment or control group would increase the sample size further. To deal with these issues we decided on a crossover design, which reduces subject variability from treatment comparisons and thus decreases the number of subjects required (Altman, 1991). In addition, this design is particularly suited for studying chronic conditions (Feingold and Gillespie, 1996). In fact the only properly controlled trial of desensitization has employed this design (Stevenson et al. 1984).

A disadvantage of the crossover trial is the possibility of a 'carry over' effect (Altman, 1991). Here the treatment effect from one period is forwarded into the next study period. Such a treatment period interaction could invalidate data obtained from the second phase of the trial. To counter this period effect a wash-out period is

included in the design of the trial. We incorporated a 1-month 'washout' phase between treatment periods (Stevenson et al. 1984).

6.1.4 Treatment dose, and regimen

We based our dose of intranasal lysine-aspirin on the Italian trial, which was 2 mgs (Patriarca et al. 1991b). However, we found that most of our patients had a positive challenge with 8-16 mgs, and required 16 mgs to be desensitized. In addition, none of our patients experienced chest symptoms at this dose during the challenge. Thus, 16 mgs was the final dose we chose for intranasal lysine-aspirin.

To determine the frequency of trial medication we considered the temporal characteristics of the refractory period (Zeiss and Lockey, 1976). These have been studied in detail (Pleskow et al. 1982). It lasts between 2-5 days. Thus, we decided to have an alternate day regimen (every 48 hours).

6.1.5 Outcome measures

Investigators have used several variables to study the treatment effect of oral or intranasal aspirin (Table 6.1). These variables can be classified as those measuring nasal changes and those measuring lung function changes. These measurements can be either subjective or objective (Table 6.2).

None of the trials to date have used objective, validated methods to study the effects of desensitization. Changes in nasal symptoms have been measured using subjective scales, which were not validated. Apart from these scales, indirect methods e.g. attack of sinusitis/year, sinus surgery/year, use of nasal corticosteroids, have been employed to indicate a change in nasal airway status. Also, in the long-term studies (Stevenson et al. 1996; Sweet et al. 1990) the final analysis and its implications depended on accurate reporting of data. Many desensitized patients were discharged to the care of their local physician who recorded subsequent changes. These methods of data entry can be a major source of variability and introduce errors in analysis. In the trial using intranasal lysine-aspirin (Patriarca et al. 1991b), the investigators did use a semi objective method to measure airway dimensions (digital rhinomanometry). However, they confined its use to the initial challenge and not in the follow up of their patients. Several studies have used measures such as

recurrence or relapse of polyps, and the need for polypectomy. These outcome measures are debatable. It is difficult to remove the entire polypoidal lining of the sinuses at surgery. This would make it difficult to document recurrence, as patients will invariably show evidence of polyps immediately after surgery if examined carefully with a rigid nasendoscope. The need for surgery can also be dependent on the patient's threshold to tolerate nasal obstruction, and the surgeon's preferences. These issues are particularly relevant in trials without any 'blinding'.

Lung function changes have been monitored using symptom scores and peak expiratory flow rate (FEV_1). Indirect methods have included documenting number of asthma attacks/year, need for hospitalisation/year, and oral or inhaled corticosteroid use. In the trial of desensitization via the nasal route (Patriarca et al. 1991b), it is mentioned that the patients' asthma did not deteriorate. However, there is no mention of the method used to monitor the lung function, and baseline versus end of trial figures are not reported. Most trials that have used oral aspirin monitored their patients' asthma using FEV_1. In the only properly controlled trial (Stevenson et al. 1984), these measurements were done on a monthly basis. This monitoring is infrequent as patients' took aspirin daily, and it could allow for small changes in lung function status to go undetected.

In designing our trial outcome measures we attempted to manage these issues by including validated methods that had been successfully used in detecting nasal and chest airway changes. Nasal airway changes were monitored using a twice-daily symptom score and NIPF. We were using the same scale for the symptom score during our intranasal lysine-aspirin challenge. It correlated well with the changes measured by acoustic rhinometry. However, the method is subjective and hence we decided to implement significance to changes in excess of 40%. NIPF is used often and has a sensitivity of 80% in detecting changes in nasal patency (Porter et al. 1996). Our primary method of detecting small changes in nasal airway patency was acoustic rhinometry (Elbrond et al. 1991b). It is highly sensitive (Porter et al. 1996), and its use as an effective tool in monitoring treatment effects on nasal polyps has been validated (Elbrond et al. 1991a; Lindholt, 1989; O'Flynn, 1993). To monitor lung function changes we decided to follow the same approach as most of the earlier trials

Table 6.2

Outcome measures used in various studies

Nasal changes		Chest changes	
Subjective	**Objective**	**Subjective**	**Objective**
Asking a patient if their nose is better/same/worse		Asking a patient if their asthma is better/same/worse	FEV_1
Symp score (scale 0-4, or 1-5)		Chest score (scale 1-5)	
Nasal CS use		Oral/Inhaled CS use	
Attacks of sinusitis/year		Hospital visits for acute attacks	
Sinus surgery/year			
Sense of smell (better/same/worse)			
Recurrence of nasal polyps			
Need for polypectomy			

Table 6.3

Outcome measures in our study

Nasal changes		Chest changes	
Subjective	**Objective**	**Subjective**	**Objective**
Daily nasal scores (validated; scale: 0-10)	Daily NIPF (am/pm)	Daily chest scores (validated; scale: 0-10)	Daily FEV_1 (am/pm)
	Acoustic rhinometry (Amin/Volume)		

and use PEFR (FEV_1). In addition, nasal/chest scores and NIPF/PEFR were measured on a twice-daily basis, making small changes detectable.

6.1.6 Critique of our clinical trial

Nine patients completed both phases of the trial, 1 completed only the lysine-aspirin phase, and 3 only the placebo phase. As mentioned in section 6.1.1 of this chapter, intranasal lysine-aspirin did not improve our patients' nasal symptoms or reduce polyp growth.

Table 6.4 summarises the changes and adds the dimension of time. It is evident from the table that there was deterioration in the nasal airway during the active phase. However, the same applies to the placebo phase and moreover the differences are not significant. This implies that patients deteriorated as expected if untreated. Also, if they were not in a desensitized state, with repeated applications of aspirin the degree of deterioration would have been greater. As this was not the case, it establishes 16 mgs as an adequate dose for desensitization.

It is important to consider the time spent by each patient in the 2 phases. Table 6.4 shows that time spent in the lysine-aspirin phase was marginally less than in the placebo phase, but the variability was higher, and a paired t-test did not show any significant difference ($p = .091$). However, a paired samples correlation was significant ($p = .011$). This indicates that each patient spent approximately the same time in the 2 phases. Again, this shows that 16 mgs of lysine-aspirin did not cause a regular nasal reaction, which would have led to a faster deterioration in these patients compared to placebo. It was also important to analyse the diary entries on a weekly basis (longitudinal data) to see if there were any period effects. We did not find any such effects or 'escape' from the desensitized state as mentioned in an earlier study (Stevenson et al. 1984).

Table 6.4 also highlights the stability of our patients' asthma status. The peak flow rate remained consistently near the baseline throughout the two trial periods, as seen in figure 5.6. None of our patients required treatment with oral corticosteroids during the trial.

Table 6.4

Changes during the trial phases

Parameter	LAS phase	Placebo phase
Time (weeks)	12.27 ± 8.73	16.27 ± 12.1
Nasal changes:		
AR - Volume	-26.14 ± 19.83	-21.24 ± 21.77
AR - Amin	-31.48 ± 39.4	-25.77 ± 51.69
NIPF	-19.73 ± 22.99	-31.93 ± 43.71
Nasal score	-32.08 ± 66.36	-38.93 ± 72.1
Chest changes:		
PEFR (FEV_1)	-2.86 ± 4.48	-3.15 ± 12.22
Chest score	-27.46 ± 34.75	-36.74 ± 120.65

AR: acoustic rhinometry

Mean ± standard deviation

Negative values indicate deterioration

6.1.7 Limitations of the trial

The main criticism of our study would be its small sample size. Our calculations suggested that we would reach an adequate power if 12 patients completed both phases successfully. To achieve this target we thought enrolling 20 patients would be sufficient. However, we fell short of our target despite enrolling 22 patients. Recruitment difficulties have been highlighted in a previous report (Stevenson et al. 1996). We found recruitment was not difficult. However, a 1-year trial period was a problem for many who were enrolled. Some patients moved away from the area and could not follow up. Others found it time consuming to maintain a regular regimen of diary keeping and medication. We realised these problems and continued to recruit but this prolonged the total duration of the trial. An ideal situation would be to enroll larger number of patients who all start at the same time, thus reducing the overall time of the trial.

6.1.8 Revised sample size calculations

Our sample size calculations (section 3.2.8) were based on results of an earlier trial (Patriarca et al. 1991a), and a formula that utilized 'effect size' in its equation (Florey, 1993). We decided to put an arbitrary figure of 50% to the effect size. This meant we were hoping to see a difference in improvement of 50% between patients on placebo and active treatment. This 50% improvement would be a composite of all outcome measures. However, in retrospect we think this is incorrect because we did not have method to convert the results into composite scores.

If the study was to be repeated we would suggest that improvement should be based on acoustic rhinometry outcome measures i.e. Volume change, and $Amin_1$ change. Based on these outcome measures we present revised figures for the sample size.

The figures are based on a nomogram used to calculate sample size (Altman, 1991). For continuous data (acoustic rhinometry measures), 4 quantities need to be specified. These are:

1. Standard deviation of variable i.e. Volume change, and $Amin_1$ change (S.D)
2. Clinically relevant difference (δ)
3. Significance level (α)
4. Power ($1 - \beta$)

We calculated the S.D for Volume and $Amin_1$ from the challenge data available to us on 98 patients. The values were 9.4 mls, and .58 mm^2 respectively. The clinically relevant difference, we think, that could be considered as a significant change would be 5 mls and .5 mm^2 respectively. Significance level and power would be set at .05 and .80 respectively. Before using the nomogram we have to calculate another quantity, which is called Standardized difference. The formula for this quantity in a cross-over trial is 2δ/S.D. Thus, the standardized difference for Volume would be 1, and that for $Amin_1$ would be 1.7. Based on these figures we use the nomogram to derive a sample size. If the primary outcome measure is volume it would be 30 patients. If the primary outcome measure is $Amin_1$ it would be 16 patients.

6.2 *Mechanism of aspirin-sensitivity*

6.2.1 Findings of laboratory-based studies

We conducted 2 laboratory-based studies to provide further insight into the mechanism of aspirin-sensitivity. The tissues studied were nasal mucosa and nasal polyps. In each study a comparison was made between aspirin-sensitive and aspirin tolerant individuals. The results of these studies have been published (Parikh et al. 2002; Sousa et al. 2002). The main findings from these studies were:

Compared to aspirin tolerant patients with nasal polyps, those with aspirin-sensitive nasal polyps express significantly more $CysLT_1$ receptors on nasal submucosal inflammatory cells. There is no difference in the expression of LTB_4 receptors.

There is a significantly higher expression of iNOS in nasal polyps from aspirin-sensitive compared to aspirin tolerant patients.

6.2.2 $CysLT_1$ receptor expression

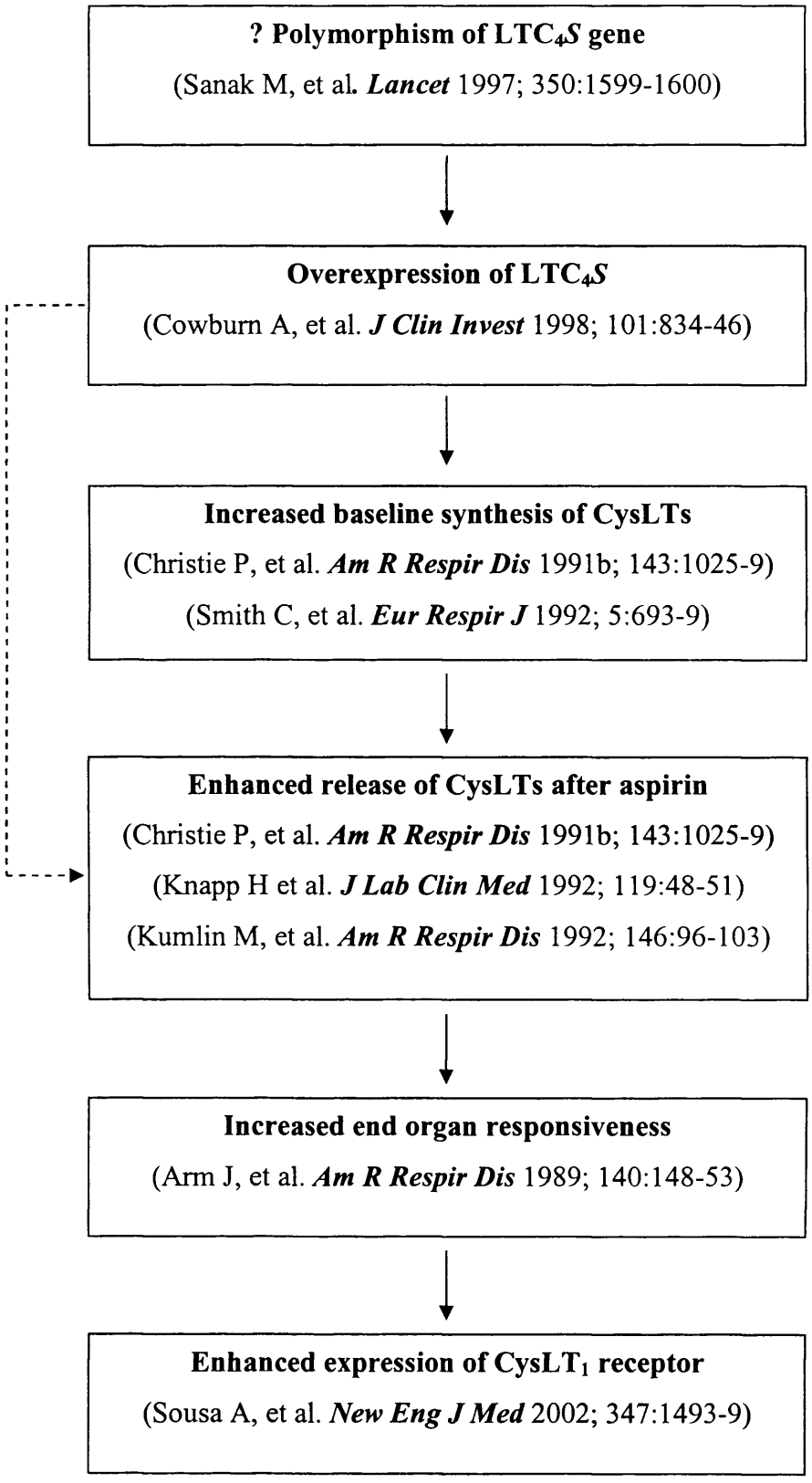
Leukotrienes, in particular cysteinyl leukotrienes, have been the primary focus of inquiry into the mechanism of aspirin-sensitivity for several years. These investigations have firmly established them as the responsible mediators of the chronic inflammatory process in aspirin-sensitive individuals.

Figure 6.1 shows the various studies that have added to the knowledge base of our understanding about the mechanism of aspirin-sensitivity. The findings of our study provide the possible reason for the increased sensitivity to cysteinyl leukotrienes.

6.2.3 Inducible nitric oxide synthase expression

As shown in figure 6.1, leukotriene production is upregulated in aspirin-sensitive patients. Cysteinyl leukotrienes have been implicated in activation of the enzyme

Figure 6.1

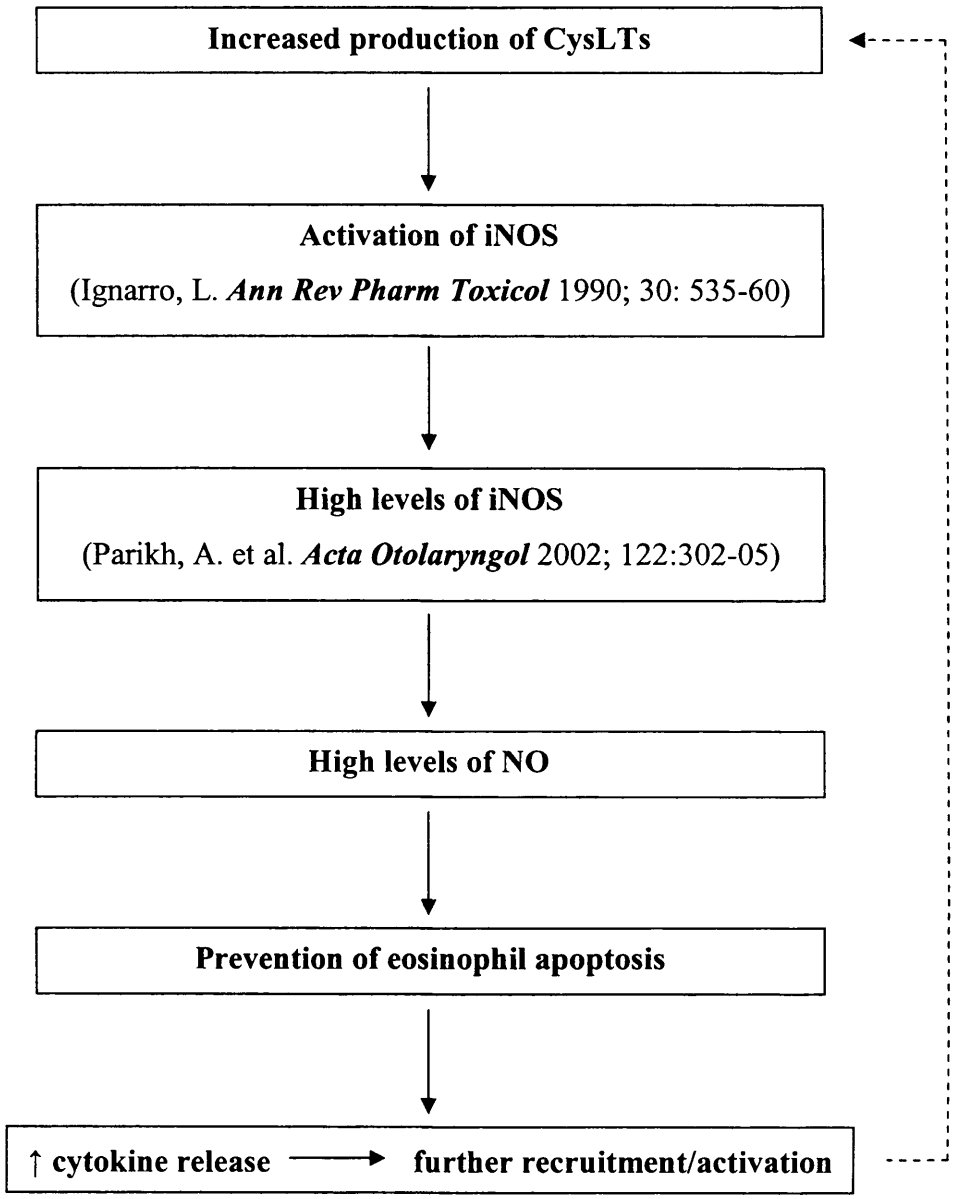

? Polymorphism of LTC4S gene
(Sanak M, et al. Lancet 1997; 350:1599-1600)
Overexpression of LTC4S
(Cowburn A, et al. J Clin Invest 1998; 101:834-46)
Increased baseline synthesis of CysLTs
(Christie P, et al. Am R Respir Dis 1991b; 143:1025-9)
(Smith C, et al. Eur Respir J 1992; 5:693-9)
Enhanced release of CysLTs after aspirin
(Christie P, et al. Am R Respir Dis 1991b; 143:1025-9)
(Knapp H et al. J Lab Clin Med 1992; 119:48-51)
(Kumlin M, et al. Am R Respir Dis 1992; 146:96-103)
Increased end organ responsiveness
(Arm J, et al. Am R Respir Dis 1989; 140:148-53)
Enhanced expression of CysLT1 receptor
(Sousa A, et al. New Eng J Med 2002; 347:1493-9)

nitric oxide synthase leading to formation of nitric oxide (Ignarro, 1990). This has been demonstrated by our study, which showed a significantly elevated expression of iNOS levels in polyp tissue of aspirin-sensitive patients as compared to aspirin tolerant individuals (Parikh et al. 2002).

We propose that these high levels of iNOS may play an important role in intensifying and self-propagating the microenvironment of inflammation in aspirin-sensitive patients (Figure 6.2). It is known that Fas ligand-Fas receptor interactions play an important role in the regulation of eosinophil apoptosis (Hebestreit et al. 1998). NO specifically prevents this interaction enhancing eosinophil survival at sites of inflammation. In aspirin-sensitive subjects this NO could prevent Fas ligand-Fas receptor interaction leading to increased survival of eosinophils, which in turn express more cytokines leading to recruitment and activation of more cells. This process could continue in an autocrine fashion propagating the inflammation. A recent study seems to confirm our suspicion that eosinophils survive longer in this microenvironment (Kowalski, 2000).

Figure 6.2

Possible role of iNOS in aspirin-sensitivity

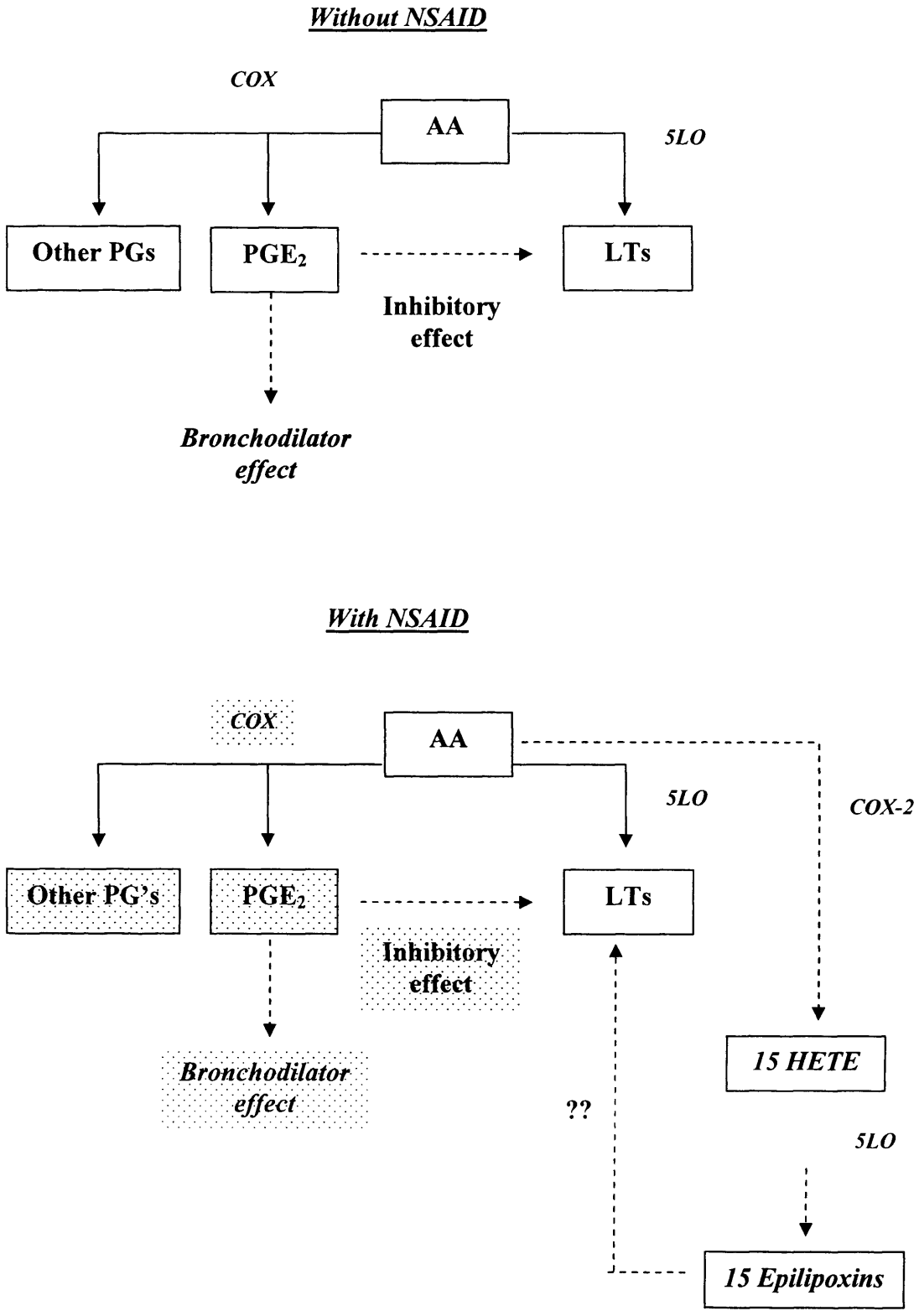


6.2.5 Role of COX-2, and COX products

Figure 6.3 explains the hypothetical role played by COX-2 and COX products in aspirin-sensitive patients, with and without intake of NSAIDs (Mitchell and Belvisi, 1997). There is scientific basis for this hypothesis. It has been shown that expression of COX-2 mRNA is enhanced in asthmatic airways of aspirin-sensitive patients (Sousa et al. 1997b). In the presence of aspirin COX-2 is modified leading to the production of 15-HETE from AA rather than prostaglandins. These metabolites are processed further by 5LO to form a new group of products called 15-Epilipoxins. It has been suggested that these novel metabolites may have a putative role in aspirin sensitivity (Mitchell and Belvisi, 1997). However, recent work suggests an anti-inflammatory role for these mediators (Chavis et al. 2000; Serhan, 2002), which does not support the above hypothesis. In addition, there is conflicting evidence about COX-2 expression in the airways of aspirin-sensitive patients. Rather than an increase some studies have shown that COX-2 expression is reduced in aspirin-sensitive patients (Picado et al. 2003; Picado et al. 1999).

Another explanation forwarded is based on the bronchoprotective role played by PGE_2. Studies have shown that inhaled PGE_2 can prevent aspirin-induced bronchoconstriction in aspirin-sensitive patients and that this may not be related to its bronchodilator action (Sestini et al. 1996; Szczeklik et al. 1996). These researchers have suggested that formation of PGE_2 probably leads to an inhibitory effect on leukotriene production and the addition of a NSAID prevents its production. Subsequently, LT production is increased due to its release from the inhibitory effect of PGE_2. This proposed mechanism has not been disputed, but fails to explain the complete picture of aspirin-sensitivity.

Figure 6.3: Role of COX products in aspirin-sensitivity

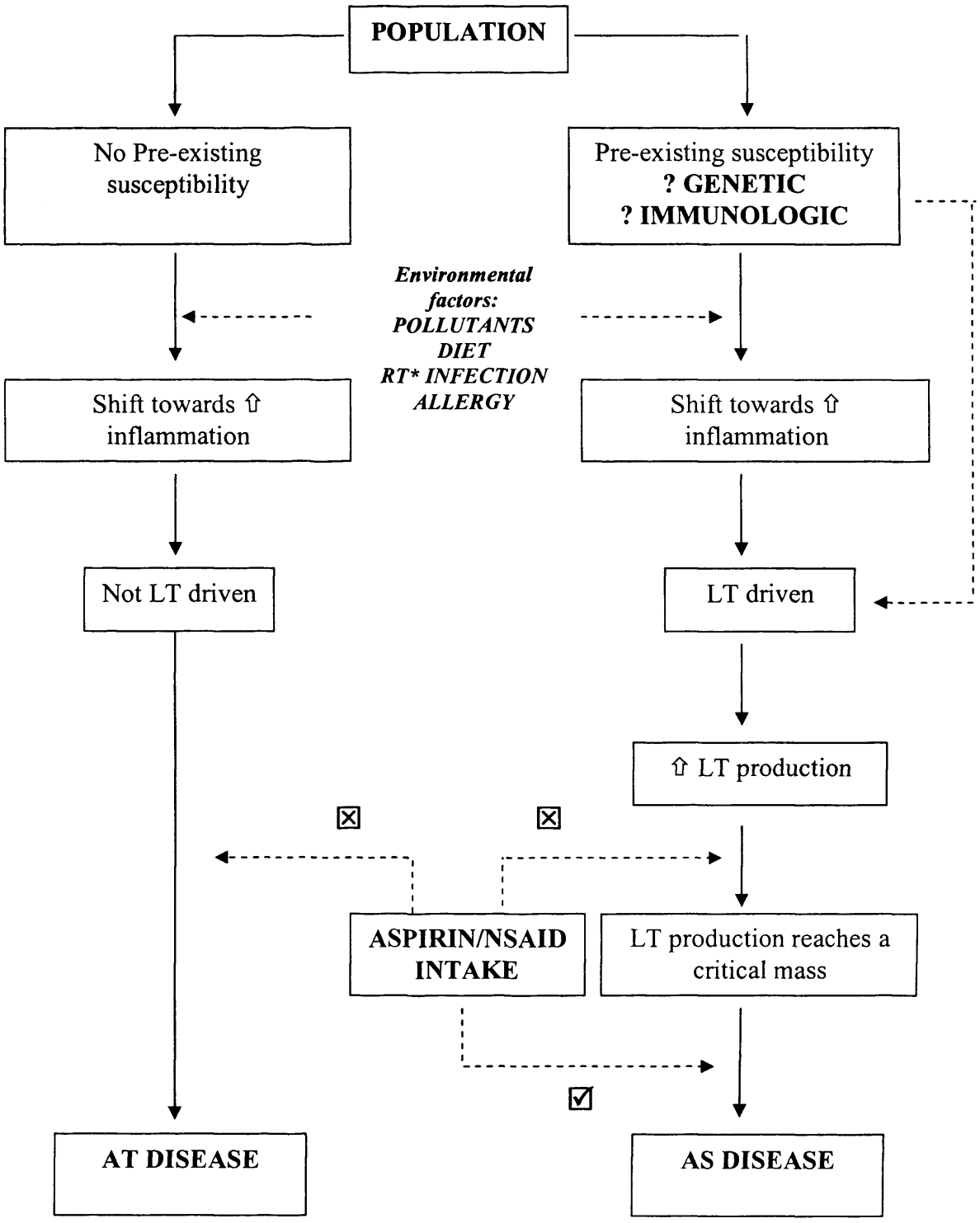


6.2.6 A new paradigm for aspirin-sensitivity

Over the years various explanations have been put forward to explain the mechanism by which aspirin and other NSAIDs cause a reaction in aspirin-sensitive individuals. As mentioned above, CysLTs seem to be at the centre of such reasoning. Despite scientific evidence of increased LT production in these individuals, their origins remain unclear, particularly in the absence of extrinsic exposure to NSAIDs. Our finding of increased $CysLT_1$ receptor expression emphasises the role of CysLTs. However, receptor physiology dictates that in most instances of chemical messenger-receptor interaction, increased levels of the chemical should lead to a down regulation of the corresponding receptor (Ganong, 1987). This is not a rigid rule e.g. angiotensin II increases the number of active receptors in the adrenal gland, or IgE increases expression of $F_c\varepsilon R$. This may be the case with CysLTs. Again, our finding of high iNOS activity helps by providing a possible explanation for reduced eosinophil apoptotic activity in aspirin-sensitive subjects.

Figure 6.4

A new paradigm for aspirin-sensitivity



RT: respiratory tract; ☑: reaction; ☒: no reaction

All these findings help our understanding of the underlying disease process at the end organ level. However, reasons for a particular individual being aspirin-sensitive in the first place remain unclear. A possible mechanism, purely speculative, which considers interplay of various factors, is shown in Figure 6.4. This hypothesis is based on the premise that a reaction to aspirin or NSAID is purely coincidental, and its intake does not play a role in the pathogenesis of this disorder. Individuals with a pre-existing susceptibility to inflammation, which is predominantly leukotriene driven are likely to develop the spectrum of manifestations when they are exposed to an interplay of various environmental factors. The pre-existing susceptibility may be genetic, immunologic, or both, ensures that the inflammation is leukotriene mediated. The individual's inherent characteristics and environmental influences continue their interplay leading to increasing leukotriene production. In the initial stages of the disease process, NSAID intake will not cause an untoward reaction, as the production of leukotriene has not reached a critical level. The inflammatory process continues driven by internal and external influences, and reaches a stage where LT production attains a critical mass. From here on if the individual takes aspirin or NSAID, a COX (1,2) blockade will remove the inhibitory influence of PGE_2, divert some metabolites towards the 5LO pathway, exaggerating the LT release, and precipitating a clinical reaction. This theory can explain the continuing inflammatory process despite lack of exposure to NSAID. However, as mentioned above, it is purely speculative.

Desensitization is more difficult to explain based on this theory. However, continued exposure to aspirin could work at different levels. Firstly, it could work as an anti-inflammatory agent. Secondly, regular intake could lead to a depletion of LT stores, and production not keeping up with its pace. Thirdly, PGE_2 production resumes 'escaping' the COX blockade. Fourthly, regular aspirin may increase production of 15-Epilipoxins that may have a beneficial effect. Lastly, as our study has shown a reduction in LT receptor expression plays an important role in desensitization (section 6.3).

6.2.7 Genetic susceptibility: role of leukotriene related gene polymorphisms

As mentioned in the previous section one possible reason for an individual to overproduce leukotrienes and develop aspirin-induced disease is their genetic

susceptibility. The enzymes involved in the production of leukotrienes are 5 lipoxygenase (ALOX5), and leukotriene C_4 synthase (LTC_4S). These have been the focus of several genetic studies. In particular, attention has been on the promoter region of the enzyme gene.

A single nucleotide polymorphism (SNP) in the LTC_4S gene has already been mentioned in section 1.2.6b. This SNP has been shown to have a positive association with aspirin induced disease in the Polish population (Sanak et al 1997, Sanak et al 2000). However, this association has not been replicated in the Japanese (Kawagishi et al 2002), American (Van Sambeek et al 2000), and Korean (Choi et al 2004) populations suggesting a population stratified finding.

Two studies have investigated promoter segment polymorphism of the ALOX5 enzyme gene (In et al 1997, Choi et al 2004). The first study showed a promoter segment polymorphism, which consisted of a variable number of tandem repeats of GC rich motifs that are associated with binding of Sp1 transcription factors (In et al 1997). Subjects with the wild type genotype (5 repeats) show a significantly higher capacity to produce CysLTs compared to a mutant genotype (3, 4 or 6 repeats). Also, the study suggested that this polymorphism may be responsible for the variable response of asthma patients to drugs that modulate the 5LO pathway.

The second study investigated the role of several leukotriene related single nucleotide polymorphisms in a group of Koreans which consisted of 93 aspirin intolerant asthmatics, 181 aspirin tolerant asthmatics, and 123 normal controls (Choi et al 2004). They found the frequency of ALOX5-ht1 (G-C-G-A) haplotype to be significantly higher in patients with aspirin-induced asthma as compared to patients with aspirin tolerant asthma (odds ratio = 5).

6.3 *Mechanism of desensitization*

Table 6.5 highlights the various changes noted following chronic aspirin intake. Apart from our study (Sousa et al. 2002), changes in nasal mucosa have never been studied before, and in particular those after long-term intranasal aspirin use. Only one study, which demonstrated a beneficial effect of intranasal lysine-aspirin has speculated on its potential mechanism (Nucera et al. 2000). They hypothesize that the effect of intranasal LAS is most likely due to its direct anti-inflammatory action. They base this on the findings of a study where LAS was shown to inhibit growth of nasal polyp cells in a culture medium (Bruzzese et al. 1998).

None of the studies using the oral route for desensitization have investigated nasal mucosal changes. However, changes shown after chronic oral aspirin use include a reduction of cysteinyl leukotriene - LTB_4, LTC_4, and LTE_4 release in nasal secretions, urine, or peripheral leukocytes (Ferreri et al. 1988; Gosepath et al. 2001; Juergens et al. 1995; Nasser et al. 1995) (Table 6.5). Also, a significant decrease in sensitivity to inhaled LTE_4 has been demonstrated (Arm et al. 1989).

These changes establish that there is a generalised down regulation of leukotriene synthesis, in particular cysteinyl leukotrienes, and also decreased end organ responsiveness. This should lead to a reduction in tissue inflammation, with improvement in nasal symptoms and slowing of polyp growth.

6.3.1 What our study adds

Using immunohistochemistry, we have shown for the first time that compared to placebo, alternate day intranasal lysine-aspirin (16 mgs) significantly reduces the number of nasal submucosal inflammatory cells expressing $CysLT_1$ receptors. This reduction seems to occur as early as 2 weeks after starting intranasal treatment, and is maintained for as long as 6 months. A significant decrease in end organ responsiveness has been demonstrated (Arm et al. 1989), and it was speculated that this is likely to be due to down regulation of the relevant receptors. Our study seems to provide this vital proof.

Speculations on the possible mechanisms of desensitization have been proposed in section 6.2.6, under 'A new paradigm for aspirin-sensitivity'.

Table 6.5

Observations on mechanism of desensitization

Authors (route, dose)	**Year**	**Observed changes after desensitization**
Ferriri et al (oral, 650 mgs)	1988	*Nasal secretions:* • Marked reduction in levels of **LTC_4** • Marked reduction in levels of **histamine**
Arm et al (oral, 600 mgs)	1989	• Marked reduction in sensitivity to inhaled **LTE_4**
Juergens et al (oral, 650 mgs)	1995	*Peripheral monocytes:* • Reduced synthesis of **LTB_4**
Nasser et al (oral, 600 mgs)	1995	*Urine:* • Reduction in mean levels of **LTE_4**
Gosepath et al (oral, 100 mgs)	2001	*Mixed lymphocyte culture:* • Reduction in release of peptido-leukotrienes, **LTB_4, LTC_4, LTE_4**
Sousa et al (intranasal, 16 mgs)	2002	*Nasal tissue:* • Marked reduction in submucosal inflammatory cells expressing **$CysLT_1$ receptor**

6.4 *Intranasal lysine-aspirin in aspirin tolerant patients with nasal polyposis: clinical trial*

6.4.1 Primary Aim

We conducted a randomised, double blind, placebo controlled, parallel group trial in aspirin tolerant patients to study the effect of intranasal lysine-aspirin on their nasal symptoms, and polyp growth. Patients used their trial medication in conjunction with regular daily intranasal corticosteroid.

We did not find intranasal lysine-aspirin to be more effective than placebo in augmenting the response to intranasal corticosteroids. Both patient groups gradually deteriorated. This was reflected by an increase in nasal symptom scores, quality of life scores, and an increase in polyp size. Quality of life deteriorated more significantly in the lysine-aspirin group. For all other measured parameters, the differences were not significant.

6.4.2 Rationale for the study

Nasal polyps are manifestations of a chronic inflammatory process for which we do not have a cure. Control of symptoms, primarily nasal blockage requires medical treatment, which consists of oral corticosteroids in bursts, and regular intranasal corticosteroids for maintenance. If these fail, surgery is the only other option. Medical therapy is aimed at the underlying inflammation and hence impacts on all symptoms including nasal obstruction, whereas surgery deals primarily with the later. Thus, any additional medical therapy would add to our armamentarium when used alone or in conjunction with intranasal corticosteroids.

There have been only 3 studies evaluating intranasal lysine-aspirin in the treatment of patients with aspirin tolerant nasal polyps (Table 6.7). Two of them have been conducted by the same group, but at different times, and with differing dose regimens (Nucera et al. 2000b; Patriarca et al. 1991b). Both concluded that intranasal lysine-aspirin was beneficial in controlling nasal polyp growth and reducing their relapse rate. The third study, which was more scientific and used objective parameters to

Table 6.6

Trials of intranasal LAS in aspirin tolerant patients

Group	Year	Type of study	Dose (mgs)	Regimen	n=; (controls)	Period (mths)	Outcome measures	Results
Patriaca et al	1991	Prospective, with controls	2	Once/week	15 (61)	24	Relapse of polyps	Relapse rate significantly less
Scadding et al	1995	Prospective, self control (opposite nostril acted as control)	2	Once/week	20 (20)	15	Symptom score Acoustic rhinometry Rigid nasendoscopy	Less polyp tissue on LAS side
Nucera et al	2000	Prospective, with controls	4	6 times/week	62 (61)	72	Recurrence of nasal polyps	Relapse rate significantly less

measure nasal symptoms and polyp growth, concluded that intranasal lysine-aspirin was possibly beneficial (Scadding et al. 1995). It was used as a pilot for a further controlled study with a larger number of patients, which is presented in this thesis.

6.4.3 Design of our study

The first study to show a beneficial effect of using intranasal lysine-aspirin in aspirin tolerant patients is open to several criticisms (Patriarca et al. 1991b). Aspirin-sensitive and aspirin tolerant subjects were grouped together, and it is unclear if the control group (n=191) had nasal challenges to characterize their aspirin-sensitive status. The control group did not receive any medication (nor placebo). Thus, the trial subjects, and investigators were not blinded. A further long-term study by the same group (Nucera et al. 2000) is also open to the same criticisms as mentioned above.

To address these issues we decided to conduct a randomised, double blind, placebo controlled, parallel group trial, which was based on an earlier pilot study (Scadding et al. 1995).

6.4.4 Treatment, dose, and regimen

We decided to use the same regimen as our pilot (Scadding et al. 1995), and the original trial that showed intranasal LAS to control polyp growth in aspirin tolerant patients (Patriarca et al. 1991b). Our subjects had to make up the solution to be instilled in their nose, and compliance was excellent with a once a week regimen. We adhered to using 16 mgs, as this was the top dose to which most aspirin-sensitive patients reacted, and a non-reaction was a good confirmation of their aspirin tolerant status. In the pilot, the trial medication was instilled into one nostril with the opposite one acting as its control. This was thought to be a potential source of bias due to cross contamination. Thus, we decided on a parallel group design with instillation of trial medication in both nostrils. Hence, the number of patients recruited was increased to a total of 40.

6.4.5 Outcome measures

Apart from our pilot study, the other 2 trials have not used objective measurements to study outcomes (Table 6.7). They used measures such as recurrence or relapse of polyps (Nucera et al. 2000b; Patriarca et al. 1991b). I have criticised the use of such measures in section 6.1.5. The same arguments apply to this trial.

In our trial we used 3 monthly symptom scores, PEFR, NIPF, rigid nasendoscopy, acoustic rhinometry, and UPSIT. All these parameters are validated methods that had been successfully used in detecting nasal and chest airway changes.

In addition, we measured quality of life changes over the period of the trial. Traditional study endpoints have been physiologic and biologic assessments, but they do not always correlate with the patient's perceptions of changes in their health status. This applies particularly to chronic ailments where surgical or medical interventions do not afford a cure, and therapy needs to be prolonged. In such instances, quality of life is the essential outcome that requires to be studied (Berzon, 1998). Most health professionals dealing with nasal polyposis are aware of its negative impact on the day-to-day lives of patients. Thus, any trial investigating a therapeutic regime should aim to measure relevant aspects of a patient's general health in addition to clinical and physiologic parameters. To implement this recommendation of the Task Force on Rhinosinusitis (Leopold et al. 1997), we included a QOL questionnaire that was completed at the patients 3 monthly visit.

6.4.6 Critique of our clinical trial

Thirty-five patients completed the trial (LAS group = 16; placebo group = 19). The follow-up period for both groups was similar (LAS group= 13.3±8.9; placebo group= 15.8±9.8, p=.36). Thus, intranasal LAS did not influence polyp growth. However, there was a steady deterioration in nasal symptoms and an increase in polyp size in both groups. This would indicate that intranasal corticosteroids slow polyp growth, but do not abolish it completely. Consequently, any adjunctive therapy that would help further would be useful.

Despite the similar deterioration in clinical parameters observed in both groups, QOL scores were significantly worse in the intranasal LAS group (Figure 5.11). This is difficult to explain. Further examination of the results (Figures 5.9, 5.10) shows

that for all measured parameters, patients in the LAS group were marginally better at baseline. This comparison is shown in Table 6.8. Also, males predominated in the LAS group. These differences are not significant on statistical analysis. However, it is possible that these may be significant if larger number of candidates were enrolled in the trial. Consequently, we speculate that patients who are better at baseline notice deterioration more, and this is reflected in their QOL scores.

6.4.7 Limitations of the trial

The limitations of this trial are very similar to those that are outlined in section 6.1.7, which addresses our trial in aspirin-sensitive patients. Despite our sample size calculations we think that the enrolled numbers fall short and fail to provide us with a more robust conclusion. Also, most anti-inflammatory medications are used on a daily basis. Our regimen was a weekly dose of LAS, and this may have been inadequate. It is difficult to change the protocol during a trial, and hence we adhered to our original regimen. An everyday or alternate day dose may have been a better option. Also, the twice-daily use of topical corticosteroid was probably maximally effective in reducing cytokine release and the addition of intranasal LAS did not have any further anti-inflammatory effects. Patients with aspirin tolerant nasal polyps do not have raised leukotrienes or leukotriene receptors, and the possibility of aspirin acting via their reduction does not arise.

Table 6.7

The two groups at baseline

Parameter	LAS group (mean±S.D)	Placebo group (mean±S.D)
Visual analogue scale	25.1±12.3	28.6±16.2
PEFR	521.2±93.5	485.5±101.8
NIPF	168.8±67.7	136.8±61.5
UPSIT	16.9±8.9	15.1±9
Endoscopy score	1.6±.9	1.9±.8
Polyp grade (L)	.9±.4	1.1±.8
Polyp grade (R)	.9±.5	1.4±.6
Acoustic rhinometry:		
Volume	22.2±7.9	19.9±10.4
$Amin_1$	1.4±.5	1.3±.5
$Amin_2$	3.1±1.7	2.7±2
Sex: M(F)	17(1)	9(10)

CONCLUSIONS AND FURTHER RESEARCH

7.1 *Intranasal lysine-aspirin in aspirin-sensitive nasal polyp disease*

7.1.1 Clinical trial

The primary aim of this study was to investigate the effectiveness of low dose intranasal lysine-aspirin (LAS) in controlling polyp growth in patients with aspirin-sensitive nasal polyposis. To achieve this we designed a randomised, double blind, placebo controlled, crossover trial. Our subjects used 16 mgs of LAS or placebo on alternate days. Measurement of polyp size was undertaken using validated methods, which included acoustic rhinometry, and nasal inspiratory peak flow. Their lung function was monitored by measuring peak expiratory flow rate.

Our results indicate that 16 mgs of LAS on alternate days does not reduce polyp growth compared to placebo. Measurement of nasal volume (0-7 cms), nasal cross-sectional area, nasal inspiratory peak flow, and nasal symptom scores indicated a gradual deterioration in both groups. The rate and magnitude of increase in polyp size was similar in both groups. There was no deterioration in lung function, which proves the long-term safety of alternate day intranasal LAS (16 mgs) instillation.

Nasal reaction to intranasal LAS was abolished by its repeated instillation, doubling the dose until 16 mgs could be applied without any adverse effect. This proves that topical intranasal application of LAS can be an alternative to oral aspirin in nasal desensitization. It is possible that this route may only affect the nasal lining, and could be used for nasal disease but may not prove beneficial in patients with accompanying systemic disease such as aspirin-sensitive asthma and urticaria.

7.1.2 Tissue studies

Our secondary aim was to compare tissue from aspirin-sensitive and aspirin tolerant individuals, which would provide an insight into the pathogenesis of aspirin-sensitive disease. Nasal mucosa biopsies were studied using immunohistochemistry. The results show that $CysLT_1$ receptor expression on submucosal inflammatory cells is significantly higher in aspirin-sensitive as compared to aspirin tolerant individuals. In addition, compared to placebo, regular alternate day intranasal LAS (16 mgs)

significantly reduces $CysLT_1$ receptor expression. This explains, at least in part, the mechanism of desensitization. Also, it confirms 16 mgs of LAS as a dose that results in changes at a molecular level although these do not translate into clinical improvement.

Apart from nasal mucosa, we studied polyp tissue and compared blood for leukotriene production in aspirin-sensitive versus aspirin tolerant patients. Polyp tissue was studied for iNOS levels. This showed a significantly higher level of iNOS in polyp tissue from aspirin-sensitive individuals suggesting a higher intensity of inflammatory change. Whole blood leukotriene production was not different amongst the two groups when it was incubated with a stimulant or with the addition of various NSAIDs.

7.2 *Intranasal lysine-aspirin in aspirin tolerant nasal polyp disease*

7.2.1 Clinical Trial

A further clinical trial was designed to explore the therapeutic benefit of once weekly topical LAS in patients with aspirin tolerant nasal polyposis. Patients were randomised to receive placebo or 16 mgs of LAS intranasally, once a week. Placebo or LAS was used in conjunction with a topical corticosteroid. We did not find any therapeutic benefit in adding topical LAS to a corticosteroid. All monitored parameters showed that nasal polyps increased in size. This deterioration was seen in both active and placebo groups. Analysis of the quality of life questionnaire showed that overall deterioration was worse in the topical LAS group.

7.3 *Further Research*

Our immunohistochemistry studies have thrown light on two issues pertaining to patients who have aspirin-sensitive nasal polyp disease. These patients have a significantly higher expression of $CysLT_1$ receptors on their nasal mucosal leukocytes, and regular alternate day intranasal lysine-aspirin (16 mgs) seems to significantly reduce this expression. The latter finding is highly suggestive of the mechanism by which patients achieve desensitization. However, this does not

translate into clinical benefit. This raises various questions that could be addressed by further research.

7.3.1 Dose of LAS

It is possible that 16 mgs of LAS on alternate days may not be sufficient to see a clinical benefit i.e. it may not result in arresting polyp growth. Hence, further work could be undertaken using a higher dose. Also, LAS could be used on a daily basis rather than every 48 hours.

7.3.2 A patient tailored approach

It is highly likely that individual patients behave differently following desensitization. In some cases it may be necessary to increase the dose of LAS after a period of time if monitored parameters show that there is some deterioration. This could prove difficult in a trial scenario due to the criteria laid down at its onset.

7.3.3 Multicentre trials

Trial based research in aspirin sensitive patients can prove to be difficult as a single institution may not be able to recruit an adequate number of subjects. In addition, withdrawals and dropouts will reduce the sample size further. Hence, multicentre trials should be designed to provide sufficient power to future studies.

7.3.4 Tissue studies following oral aspirin desensitization

Clinical benefit following oral aspirin desensitization has been shown in various trials as outlined in chapter 1. However, tissue studies have not been undertaken. It would be of interest to examine nasal tissue to see if there is a reduction in expression of $CysLT_1$ receptors. If that were not the case it would indicate that this phenomenon is restricted to topical LAS use, and that there is a further mechanism of desensitization at work leading to improvement in nasal symptoms. Also, polyp tissue could be analysed in these patients to see if iNOS levels reduced.

7.3.5 Pathophysiology

Enhanced expression of $CysLT_1$ receptors adds to our understanding of this disease process. However, the underlying reason for a hyper-functioning leukotriene pathway down to the receptor level remains unknown. I have speculated on the possible reasons for this in section 6.2.6. These could involve genetic and environmental factors. A large database, and collaborative work will help to shed further light on these issues.

Our study on whole blood did not show overproduction of leukotrienes when incubated with a stimulant or NSAIDs. However, further work on polyp tissue or bronchial biopsies should be performed as it may show that this phenomenon is localised to the respiratory tract mucosa. This switch may occur due to a virus confined to the respiratory tract, and a study aimed at isolating it would improve our understanding of this disease process. Also, it is possible that patients who exhibit a more generalised reaction to aspirin intake such as urticaria, angioedema, or anaphylaxis may have increased blood leukotriene production. To investigate this possibility it would be important to have rigid inclusion criteria isolating such individuals that would be included in the study. If increased blood leukotriene production was found in these patients a different mechanism for aspirin sensitivity would have to be considered in these patients.

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APPENDICES

Appendix A

Confidential

CONSENT FORM

Study Title Double blind, placebo controlled, trial of intranasal lysine-aspirin, in aspirin-sensitive patients with nasal polyposis and asthma.

Investigators Dr. Glenis Scadding
Mr. Abhi Parikh

To be completed by patient/volunteer

	Delete as necessary
1. Have you read the information sheet about this study?	YES/NO
2. Have you had an opportunity to ask questions and discuss this study?	YES/NO
3. Have you received satisfactory answers to all your questions?	YES/NO
4. Have you received enough information about this study?	YES/NO
5. Which doctor have you spoken to about this study?	Dr.....
6. Do you understand that you are free to withdraw from this study? * at any time * without giving a reason for withdrawing * without affecting your medical care ?	YES/NO
7. Do you agree to take part in this study?	YES/NO

Signed ..... Date .....

Name in block letters .....

Doctor .....

Appendix B

INFORMATION SHEET

Study Title Double blind, placebo controlled, cross-over trial of intranasal lysine-aspirin, in aspirin-sensitive patients with nasal polyposis and asthma.

Investigators Dr. Glenis Scadding, Consultant Physician in Clinical Immunology, Allergy & Rhinology.
Mr. Abhi Parikh MS FRCS, Research Fellow

Hospital Royal National Throat, Nose & Ear hospital, Gray's Inn road, London WC1X 8DA

You are being requested to take part in the above named study. It will help us to find out the effectiveness of using aspirin in the nose to control your nasal polyps and asthma. We would request good compliance and co-operation if you decide to take part in the project. The fundamental idea is that regular small doses of aspirin in the nose can "desensitize" the nose, and possibly the lungs. It is essential that the treatment is taken REGULARLY every other day. If you miss two doses, DO NOT take the next one, but contact us on 0171 915 1674.

The study will run over 12 months. You will undergo a polypectomy at the start. 2 weeks following your operation you will be enrolled for the study. This will entail taking lysine-aspirin or placebo (salt solution), on alternate days for a period of 6 months. At the end of this period you will enter a 'washout' phase where neither of the above drugs will be used (a 'rest' period). Then we will switch you into the second part of the study using either placebo or lysine-aspirin.

During each 6 month period you will be asked to come for assessments at intervals of 6 weeks. These will be brief and will include measuring your polyp growth and nasal area. We will use a telescope to view the inside of your nose. A special instrument will be used to measure the area inside your nose. None of these assessments involve any pain. You will be asked to maintain a diary card during this period. Clear instructions will be mentioned on the diary card.

At the start of the study and at the end of each 6 month period, we will challenge your nose with lysine-aspirin and collect a nasal lavage sample for laboratory analysis. At the same time a small biopsy will be taken under local anaesthetic. This may cause slight discomfort but should not be unduly painful.

This study should help you and similar patients by reducing the number of polyp operations, and in controlling their asthma.

We do not have external funding for this trial as yet, but can pay traveling expenses. So please bring your tickets with you at each visit.

You do not have to take part in this study if you do not want to. If you decide to take part you may withdraw at any time without having to give a reason. Your decision whether to take part or not will not affect your care and management in any way.

Appendix C

INSTRUCTIONS TO PATIENTS

How to prepare the drops to be put in the nose.

You are provided with:

1. **CAPSULES**; which contain powder
2. **BOTTLE**; of liquid (the diluent)
3. **MEASURING CUP**
4. **STIRRER**; plastic
5. **DROPPER**

You need to have ready:

1. Pair of sharp SCISSORS
2. Clean tea spoon (if stirrer not provided)

Steps to prepare drops:

1. Pour liquid into cup to the level marked 10, and place on table.
2. Take a capsule and flick it while holding straight up. The powder should settle leaving an air space above.
3. Using the scissors, snip off the top of the capsule where the air space is.
4. Empty the capsule powder into the cup. Flick if necessary so that the capsule has been emptied.
5. Stir the powder in the liquid using the stirrer or the spoon. The powder should dissolve quickly.
6. Load the dropper with the solution.
7. Place 2 drops in each side of the nose, holding the head as has been shown to you.
8. Throw away the remaining solution. It must not be kept for the next time.
9. Do this every other day i.e. alternate days.

Appendix D

DIARY CARD

NAME: **TRIAL NO:**

START DATE:

NEXT VISIT:

INSTRUCTIONS:

To complete symptom score, please score each symptom on a **0 to 9** basis where:

0 = no symptoms
9 = worst possible symptoms

Symptoms to consider for nasal scoring:
blockage, running, itching, sneezing, sense of smell

Symptoms to consider for chest scoring:
tightness of chest, wheeze, shortness of breath on exertion, daytime cough, night cough

PEFR = highest of 3 readings
NIPF = highest of 3 readings

Contact Telephone no.:

WEEK NO. :

DATE							
	AM	AM	AM	AM	AM	AM	AM
NASAL SCORE							
CHEST SCORE							
PEFR							
NIPF							
	PM	PM	PM	PM	PM	PM	PM
NASAL SCORE							
CHEST SCORE							
PEFR							
NIPF							
TRIAL DRUG (ALT. DAYS)							
OTHER DRUGS							

Appendix E (reproduced)

Name of patient

Date

Pre:

Challenge with:

NIPF

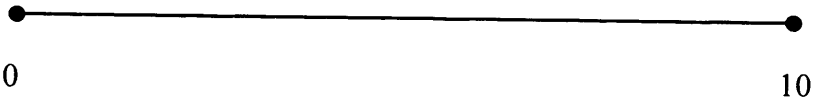
PEFR

Rhinometry

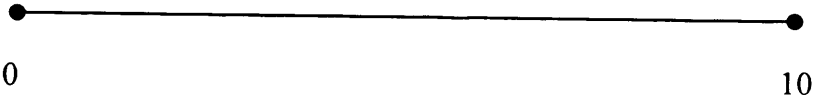
VAS

Sneeze

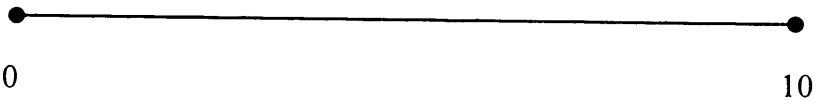
Itch



Obstruction



Running (Ant+Post)



Appendix F

Confidential

CONSENT FORM

Study Title Double blind, placebo controlled, trial of intranasal lysine-aspirin in addition to regular topical corticosteroids, in aspirin tolerant patients with nasal polyposis .

Investigators Dr. Glenis Scadding Consultant Physician in Clinical Immunology, Allergy & Rhinology.
Mr. Abhi Parikh MS FRCS, Research Fellow

To be completed by patient/volunteer

	Delete as necessary
1. Have you read the information sheet about this study?	YES/NO
2. Have you had an opportunity to ask questions and discuss this study?	YES/NO
3. Have you received satisfactory answers to all your questions?	YES/NO
4. Have you received enough information about this study?	YES/NO
5. Which doctor have you spoken to about this study?	Dr.....
6. Do you understand that you are free to withdraw from this study? * at any time * without giving a reason for withdrawing * without affecting your medical care ?	YES/NO
7. Do you agree to take part in this study?	YES/NO

Signed ..... Date .....

Name in block letters .....

Doctor .....

Appendix G

INFORMATION SHEET

Study Title	Double blind, placebo controlled, trial of intranasal lysine-aspirin in addition to regular topical corticosteroids, in aspirin tolerant patients with nasal polyposis .
Investigators	Dr. Glenis Scadding, Consultant Physician in Clinical Immunology, Allergy & Rhinology. Mr. Abhi Parikh MS FRCS, Research Fellow
Hospital	Royal National Throat, Nose & Ear hospital, Gray's Inn road, London WC1X 8DA

You are being requested to take part in the above named study. It will help us to find out if aspirin (in the form of a nasal spray) in addition to your regular treatment is helpful in controlling your nasal polyps. We would request good compliance and co-operation if you decide to take part in the project.

The study will run over 12 months. It is a randomized study and hence you have a 50% chance of receiving aspirin or placebo (salt solution). The medication will be in the form of capsules from which you will prepare a solution to be used as a spray. This has to be used once a week. Every week you have to make a fresh preparation. This treatment is in addition to daily Flixonase in nebule form.

During the 12 month period you will be asked to come for 3 monthly assessments. These will include measuring your polyp growth and nasal area. We will use a telescope to view the inside of your nose. A special instrument will be used to measure the area and volume inside your nose. None of these assessments involve any pain. You will be asked to maintain a diary card of your daily nasal symptoms and airflow measurements during this period. Clear instructions will be mentioned on the diary card.

You do not have to take part in this study if you do not want to. If you decide to take part you may withdraw at any time without having to give a reason. Your decision whether to take part or not will not affect your care and management in any way.

Appendix H

DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL OF INTRANASAL LYSINE-ASPIRIN IN ADDITION TO CORTICOSTEROIDS IN THE CONTROL OF NASAL POLYPOSIS IN ASPIRIN-TOLERANT PATIENTS

Patient name:	
Patient Hospital no:	
Patient Trial no:	

Visit 1

Date:

Check if the patient fulfills these inclusion/exclusion criteria.

Inclusion criteria:

1. Adults >18 years
2. Aspirin tolerant
    - on history
    - negative aspirin challenge
3. Nasal polyps

Exclusion criteria:

1. Aspirin-sensitivity
2. Acute Infective Rhinitis (at present or in the 2/52 preceding)
3. Severe deviation of nasal septum
4. Severe obstructive nasal polyps
5. Pregnancy/Lactation
6. Psychiatric illness

Visit 1 (continued)

	√ or x
1. Information sheet	☐
2. Consent	☐
3. Acclimatize (10-15 minutes)	☐
4. VAS (for this study)	☐
5. Peak flow (3 readings) and NIPF	☐
6. Acoustic rhinometry (same ∠, nose-piece, seat)	☐
7. QOL questionnaire	☐
8. Appointment (3/12)	☐
9. Endoscopy (0-3 scale)	☐

- polyp size
- oedema
- discharge
- crusting

10. UPSIT	☐

Visit 2 (3 months)

Date:

	√ or x
1. Acclimatize (10-15 minutes)	☐
2. VAS (for this study)	☐
3. Peak flow (3 readings) and NIPF	☐
4. Acoustic rhinometry (same ∠, nose-piece, seat)	☐
5. Appointment (3/12)	☐
6. Endoscopy (0-3 scale)	☐

- polyp size
- oedema
- discharge
- crusting

7. UPSIT	☐

Visit 3 (6 months)

Date:

	√ or x
1. Acclimatize (10-15 minutes)	☐
2. VAS (for this study)	☐
3. Peak flow (3 readings) and NIPF	☐
4. Acoustic rhinometry (same ∠, nose-piece, seat)	☐
5. QOL questionnaire	☐
6. Appointment (3/12)	☐
7. Endoscopy (0-3 scale)	☐

- polyp size
- oedema
- discharge
- crusting

8. UPSIT	☐

Visit 4 (9 months)

Date:

	√ or x
1. Acclimatize (10-15 minutes)	☐
2. VAS (for this study)	☐
3. Peak flow (3 readings) and NIPF	☐
4. Acoustic rhinometry (same ∠, nose-piece, seat)	☐
5. Appointment (3/12)	☐
6. Endoscopy (0-3 scale)	☐

- polyp size
- oedema
- discharge
- crusting

7. UPSIT	☐

Final visit (12 months)

Date:

	√ or x
1. Acclimatize (10-15 minutes)	☐
2. VAS (for this study)	☐
3. Peak flow (3 readings) and NIPF	☐
4. Acoustic rhinometry (same ∠, nose-piece, seat)	☐
5. QOL questionnaire	☐
6. Appointment (3/12)	☐
7. Endoscopy (0-3 scale)	☐

- polyp size
- oedema
- discharge
- crusting

8. UPSIT	☐
9. Thank patient	

Drop-out visit or if patient is withdrawn

Date:

	√ or x
1. Acclimatize (10-15 minutes)	☐
2. VAS (for this study)	☐
3. Peak flow (3 readings) and NIPF	☐
4. Acoustic rhinometry (same ∠, nose-piece, seat)	☐
5. QOL questionnaire	☐
6. Appointment (3/12)	☐
7. Endoscopy (0-3 scale)	☐

- polyp size
- oedema
- discharge
- crusting

8. UPSIT	☐
9. Thank patient	

Appendix I

DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL OF INTRANASAL LYSINE-ASPIRIN IN ADDITION TO CORTICOSTEROIDS IN THE CONTROL OF NASAL POLYPOSIS IN ASPIRIN-TOLERANT PATIENTS

Symptom scale

These markings are for your symptoms in the **past 1 week only**. ***PLEASE CIRCLE A NUMBER.***

Name: **Date:**

0 = **NO TROUBLE** WITH THE SYMPTOM
10 = **SEVERE TROUBLE** WITH THE SYMPTOM

- Difficulty in breathing through the nose (Nasal blockage)

GOOD 0 1 2 3 4 5 6 7 8 9 10 **BAD**

- Runny nose (discharge from the nostril)*

0 1 2 3 4 5 6 7 8 9 10

- Discharge going down the back of your nose (postnasal drip)*

0 1 2 3 4 5 6 7 8 9 10

- Sneezing

0 1 2 3 4 5 6 7 8 9 10

- Headaches

0 1 2 3 4 5 6 7 8 9 10

- Pain around the eyes/cheek (facial pain)

0 1 2 3 4 5 6 7 8 9 10

- Sense of smell

NORMAL 0 1 2 3 4 5 6 7 8 9 10 **NO SMELL**

*For discharge indicate colour by circling:

- C = clear
- D = green, yellow

Discharge from front = C or D
Discharge from back = C or D

Appendix J

QUALITY OF LIFE QUESTIONNAIRE

Name:
Patient hosp. no:
Patient trial no:
Date: Visit no:

Circle one answer per line

SLEEP

To what extent have you been troubled by each of the following problems because of your nose problems in the last week?

	not at all	very slightly	slightly	moderately	fairly	very	extremely
a. Trouble falling asleep	0	1	2	3	4	5	6
b. waking up during the night	0	1	2	3	4	5	6
c. lack of sleep	0	1	2	3	4	5	6
d. waking up feeling tired	0	1	2	3	4	5	6
e. night cough	0	1	2	3	4	5	6

OTHER SYMPTOMS

During the past week, to what degree have you been disturbed by each of the following problems, due to the nose?

	not at all	very slightly	slightly	moderately	fairly	very	extremely
a. tiredness	0	1	2	3	4	5	6
b. thirst	0	1	2	3	4	5	6
c. reduced productivity	0	1	2	3	4	5	6
d. difficulty concentrating	0	1	2	3	4	5	6
e. headaches	0	1	2	3	4	5	6
f. exhaustion	0	1	2	3	4	5	6
g. poor sense of smell	0	1	2	3	4	5	6
h. poor sense of taste	0	1	2	3	4	5	6
i. catarrh	0	1	2	3	4	5	6

NASAL PROBLEMS

During the past week, to what degree have you been disturbed by each of the following?

	not at all	very slightly	slightly	moderately	fairly	very	extremely
a. stuffy nose	0	1	2	3	4	5	6
b. runny nose	0	1	2	3	4	5	6

c. sneezing	0	1	2	3	4	5	6
d. itchy nose	0	1	2	3	4	5	6

PRACTICAL PROBLEMS

During the past week, to what degree have you been disturbed by each of the following problems, because of your nose?

	not at all	very slightly	slightly	moderately	fairly	very	extremely
a. the inconvinience of always having to carry a handkerchief	0	1	2	3	4	5	6
b. the need to rub your nose	0	1	2	3	4	5	6
c. the need to blow your nose frequently	0	1	2	3	4	5	6
d. the need to clear your throat	0	1	2	3	4	5	6
e. cough	0	1	2	3	4	5	6

ACTIVITIES

During the past week, to what degree have you been disturbed during your activities because of your nose problems? (Choose your own 3 activities - you do not have to mention the activities chosen)

	not at all	very slightly	slightly	moderately	fairly	very	extremely
a. Activity 1	0	1	2	3	4	5	6
b. Activity 2	0	1	2	3	4	5	6
c. Activity 3	0	1	2	3	4	5	6

EMOTIONAL STATE

During the past week, how often have you been in each of the following emotional states because of your nose problems?

	not at all	very slightly	slightly	moderately	fairly	very	extremely
a. Frustration	0	1	2	3	4	5	6
b. impatience and restlessness	0	1	2	3	4	5	6
c. irritability	0	1	2	3	4	5	6
d. ill at ease due to the nose	0	1	2	3	4	5	6
e. feeling low	0	1	2	3	4	5	6
f. difficulty with coping	0	1	2	3	4	5	6

PUBLICATIONS